FUNGOUS DISEASES AND THEIR TREATMENT

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FUNGOUS DISEASES AND THEIR TREATMENT

Edited by

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FOREWORD

THE Symposium whose proceedings are reported in this book was held, under the aegis of the British Postgraduate Medical Federation, at the Institute of Diseases of the Chest, Brompton Hospital, in July, 1957. It owed its origin to an offer to the Federation by Messrs E. R. Squibb and Sons to give financial support to such a symposium. An organizing committee was constituted by representatives of those Institutes of the Federation in whose special work fungous diseases are important, as follows:

Dr. J. G. Scadding (Chairman)	Institute of Diseases of the Chest
Dr R. W. Riddell .	Institutes of Diseases of the Chest and Dermatology
Dr F R. Bettley . . .	Institute of Dermatology
Dr S Gold	Institute of Dermatology
Dr N F. Norris	Institute of Obstetrics and Gynaecology
Dr A. P. Norman	Institute of Child Health
Dr G T Stewart (co-opted)	

Dr Riddell and Dr Stewart acted as Honorary Secretaries of the Committee and subsequently as Editors of the Proceedings

The Symposium brought together the experience of workers from several parts of the world The Organizing Committee, in order to encourage full and free discussion, decided to limit invitations to workers active in the study of various aspects of fungous diseases. While some fungous infections of man are widespread throughout the world, others seem to have a restricted geographical distribution Where the more serious diseases are endemic, for example, coccidioidomycosis and histoplasmosis in the United States, the locally endemic diseases have been studied intensively, and this has stimulated interest in the general problems of fungous infections At the same time, interest in these diseases is increasing in Great Britain. The importance of fungi has long been recognized in dermatology, and the clinical aspects of the more frequent systemic fungous infections, such as actinomycosis, are well known. The clinical pictures of some of the less frequent pulmonary infections, such as aspergillosis, are at present being elucidated in this country, and the widespread use of antibiotics has focused attention upon the problem of fungous super-infections in patients so treated Nevertheless,



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the therapy of fungal infections, with the exception of actinomycosis, is poorly defined, in spite of the range of agents, old and new, which have been brought forward for consideration.

The Symposium gave an opportunity, unique in this country, for defining both the present state of knowledge and outstanding problems in the study of fungi in relation to human disease. Its work was divided into sessions on pathology, under the chairmanship of Professor Robert Cruickshank; on dermatology (Dr. G. B Dowling); on medicine (Dr. J. G. Scadding); on therapeutics (Professor L. P. Garrod), on gynaecology (Professor W. C. W. Nixon); and on paediatrics (Professor A. A. Moncrieff). Discussion sessions were opened by Dr J. L Livingstone (London), Dr C. H. Whittle (Cambridge), Professor Roger Baker (Durham, N. Carolina), Mr. Aleck Bourne (London) and Professor Hans Götz (Munich)

It is fortunate indeed that it has proved possible to publish the Proceedings of the Symposium, and thanks are due to the participants who agreed not only to provide the scripts of their papers for publication (which more than doubled the labour of contributing to a symposium of this sort), but also to permit editing in order to secure some uniformity of presentation It is hoped that these Proceedings will constitute not only a useful summary of current knowledge and views concerning fungous diseases and their treatment, but also a worthy record of the collaboration which the Symposium represented between the participants from this country and overseas, the several Institutes of the British Postgraduate Medical Federation which were concerned in its organization, and the Pharmaceutical House without whose financial support it would not have been possible

J G. SCADDING

August, 1958

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Part 2

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EDITORS' PREFACE

Our aim in preparing this book has been to condense the papers read at the Symposium on Fungous Diseases into a useful work of reference. The Symposium was designed to bring together individuals with different approaches to each topic, and was characterized by contrasts in subject-matter. Thus, papers on the clinical aspects were followed by others dealing with the wider problems of industrial and epidemiological management of fungous infections, while the criteria used in the diagnosis of isolated cases were set against the background of geographical and communal incidence.

A book prepared from so many sources can hardly be systematic but it can highlight the principal advances in the subject and, equally important, emphasize the main gaps in present-day knowledge and approach On reading the various papers delivered at the Symposium, we have been impressed by the need for re-assessment of the pathogenicity of some well-known fungous species such as *Candida albicans* and *Aspergillus fumigatus*, and by the unsuspected prevalence in some communities of organisms like *Histoplasma capsulatum*. The imaginative methods recently developed, particularly in the United States, for early case detection and epidemiological study in histoplasmosis deserve special emphasis both as examples of the handling of localized incidents and as a pattern for dealing with certain other infections In contrast, it is apparent that traditional methods have done much to control many of the commoner infections of the skin and hair.

Perhaps the least rational topic in this field is that of therapy. Some contributors spoke of new chemotherapeutic agents, others, with equal conviction and authority, spoke of classical empirical therapy with dyes and tars The dermatologists in particular seemed convinced of the adequacy and even superiority of the older medicaments The modern has so often gained by re-assessment of the traditional that we have felt it a duty in this volume to give heed to both But we have also found ourselves driven to the conclusion that a more critical study was required of the mode of action of the few effective drugs used for treating mycotic diseases The treatment of ringworm infections due to anthropophilic species of dermatophytes has not altered over the years and is still inadequate, much has yet to be learnt about the treatment of the pulmonary and systemic mycoses

PART I

PATHOLOGY, CLINICAL FEATURES AND EPIDEMIOLOGY

THE ROLE OF FUNGI AS HUMAN PATHOGENS

R. W. RIDDELL

ONLY very few species of fungi which exist as saprophytes in Nature have the ability to infect man though many of them can evoke allergic reactions in hypersensitive subjects. The few which do produce disease may be considered in 3 morphological groups and this separates them broadly in their roles as pathogens.

MORPHOLOGICAL GROUPS

Filamentous fungi.—These are mostly only locally invasive, but rarely some produce disseminated infections The spores they form comprise the infective elements by which disease is acquired. Examples are the dermatophytes (or ringworm fungi) and *Aspergillus fumigatus* (Fig 1)

Yeasts.—These are unicellular fungi which reproduce by budding and which are, by virtue of their structure, relatively readily disseminated in the tissues. There is one important example, namely *Cryptococcus neoformans* (Fig 2)

Organisms which under the same cultural conditions grow both as



FIG 1 —*Aspergillus fumigatus* Mycelium and spore structure (Lactophenol blue, ×336)

yeasts and elongated filamentous cells are called *yeast-like* fungi. They are exemplified by *Candida* (*Monilia*) *albicans* (Fig. 3).

Dimorphic fungi.—This small group grow either as filaments or as yeasts according to whether the temperature of culture is approximately 22° C. or 37° C., or whether the fungus is existing as a saprophyte or parasite. These two forms do not occur simultaneously under the same environmental conditions. *Blastomyces dermatitidis* is an example (Fig. 4a and b).

In addition to these groups of true fungi, the actinomycetes are usually considered among the causative agents of mycotic diseases. They should, however, properly be classified among the bacteria. [illegible]

[illegible] acceptance of a uniform terminology for pathogenic fungi and this is depicted in the Medical Research Council Memorandum No 23[4] In summarizing the properties of fungi which produce disease in man, these organisms will be grouped according to their role as pathogens (Tables 1, 4, 5); the clinical pathology of mycotic diseases is summarized elsewhere[6, 7, 9, 10].

SKIN INFECTIONS

The dermatophytes.—The fungi which exhibit the highest degree of specialization from the point of view of parasitic habitat are those which invade the keratin of skin and its appendages (Table 1)

TABLE 1
KERATIN INVADERS

Fungus	*Disease*	*Classification*
Fungi causing superficial mycoses		
Malassezia furfur	Pityriasis versicolor	Y-L
Nocardia minutissima	Erythrasma	B
Nocardia tenuis	Trichonocardiosis axillaris	B
Piedraia species, etc	Piedra	F
Fungi causing ringworm		
Microsporum species	Tinea of skin, hair and nails	F
Trichophyton species		
Epidermophyton species		
Candida albicans	Moniliasis	Y-L

B = Bacterium, Y = Yeast, Y-L = Yeast-like fungus; F = Filamentous fungus

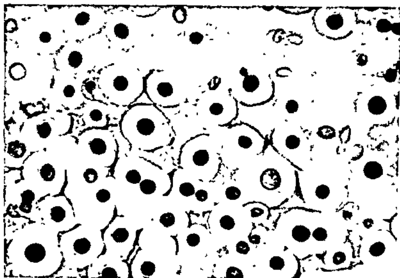


FIG 2 —*Cryptococcus neoformans* Yeast cells with surrounding capsules (Periodic acid Schiff, ×420)



FIG 3 —*Candida albicans* Yeast cells, some elongated to form pseudomycelium (Lactophenol blue, ×565)

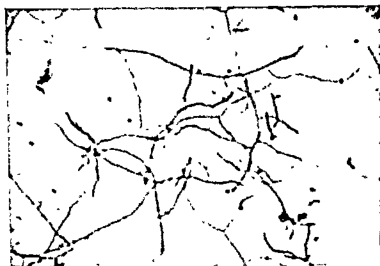


FIG 5 —*Actinomyces israeli* Fine branching mycelium (×900)

The dermatophytes are the most successful parasites from this point of view because of their ability to lyse keratin When invading skin structures they may advance to the vicinity of, but never into, the zone of living keratogenous cells (Fig 6) Distinct differences in potentiality for invasion are apparent within this closely related group of fungi, especially in connexion with the keratin of nails and hair (Table 2) *Microsporum audouini*, for example, shows a great affinity for the hair of young subjects yet it does not attack nails, *Trichophyton rubrum* on the other hand only rarely attacks hair but produces deep and intractable infection of nails

Only when hair is invaded *in vivo* by certain ringworm organisms is a fluorescent chemical material produced in amounts detectable to the naked eye under ultra-violet light (Table 2) Considerable differences are apparent also in host reactions in these infections, the most important single factor concerned being the fungous species responsible Some ringworm fungi, especially those zoophilic species which infect animals as well as man (Table 3), produce acute inflammatory lesions with erythema, vesiculation and even suppuration (Fig 7)

Other species, generally the anthropophilic strains which only very rarely infect animals, excite only a slight tissue reaction consisting of hyperkeratosis (Fig 8) and slight infiltration of the epidermis



(a)





(b)

Fig 4 —*Blastomyces dermatitidis* (a) Saprophytic phase Mycelium with single-celled spherical spores from culture grown at 22° C (Lactophenol blue, ×900) (b) Parasitic phase Yeasts from culture grown at 37 C (Lactophenol blue, ×900)



FIG 6—*Trichophyton rubrum* infection of skin Section shows fungous filaments deep in keratin layer (Periodic acid Schiff, ×350) (*By courtesy of Dr C D Calnan*)

by mononuclear cells. *Trichophyton schoenleini* is an exception to this generalization as is obvious in the clinical syndrome of favus; exceptions also occasionally occur in various other ringworm species[5]

Uncultured fungi—In contrast to the ringworm fungi, organisms like *Malassezia furfur* (Fig 9) and *Nocardia minutissima* (Fig 10) colonize only the most superficial layers of keratin and are responsible only for mildly inflammatory lesions typical of pityriasis versicolor and erythrasma respectively These fungi have not been cultured

Candida albicans—Requiring separate and special mention among the keratin invading fungi is *C albicans*, a potential pathogen which appears to need the existence of certain predisposing factors before

TABLE 2

SUSCEPTIBILITY OF HAIR AND NAIL TO INFECTION BY DERMATOPHYTE SPECIES

	Hair invasion			*Nail invasion*
	Degree	*Spore arrangement*	*Fluorescence*	*Degree*
Microsporum:				
M. audouini	++	SSE	++	−
M. canis	++	SSE	++	−
M. gypseum	±	SSE	−	−
Trichophyton:				
T. mentagrophytes	+	SSE	−	+
T. rubrum	−	−	−	++
T. verrucosum	+	LSE	−	−
T. sulphureum	++	E	−	+
T. violaceum	++	E	−	+
T. schoenleini	++	E	+	+
Epidermophyton:				
E. floccosum	−	−	−	±

SSE = Small-spored ectothrix hair infection, LSE = Large-spored ectothrix infection, E = Endothrix hair infection

TABLE 3

NATURAL HABITAT OF RINGWORM SPECIES RELATED TO TYPE OF HUMAN LESIONS

Anthropophilic species producing non-inflammatory lesions	*Zoophilic species producing inflammatory lesions*	*Soil species producing inflammatory lesions*
Microsporum species *M. audouini*	*M. canis* (cat and dog) *M. equinum* (horse)	*M. gypseum*
Trichophyton species *T. rubrum* *T. sulphureum* *T. violaceum* *T. schoenleini*	*T. mentagrophytes* (cat, cattle, dog and horse) *T. verrucosum* (cattle and horse) *T. quinckeanum* (mouse) *T. equinum* (horse)	
Epidermophyton species *E. floccosum*		

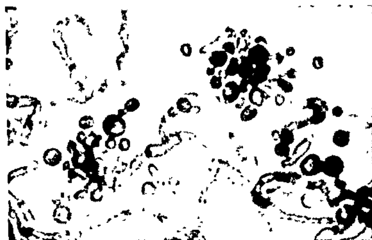


FIG 9 —*Malassezia furfur* in skin scrapings from patient with pityriasis versicolor (Periodic acid Schiff, ×900)



FIG 10 —*Nocardia minutissima* in skin scrapings from patient with erythrasma (×900)



FIG 7 —*Trichophyton verrucosum* infection of skin of forearm Contracted from cattle (*By courtesy of Dr A D Porter*)



FIG 8 —*Trichophyton rubrum* infection of palm of hand Limited to humans (*By courtesy of Dr F R Bettley*)

it can become established in skin. Maceration is probably the most important of these, and prior antibiotic therapy and diabetes are others. Nails are very rarely invaded by this fungus and hairs never. Compared with most fungi infecting skin, it is an inefficient parasite but, unlike other skin pathogens, it also infects mucosae and, on rare occasions, produces disseminated disease.

Parasitic and saprophytic comparison.—The dermatophytes are markedly inhibited in their parasitic life in skin compared with their saprophytic growth in culture. *In vivo* in keratin, hyphae segmenting into chains of arthrospores are formed (Fig. 11b) whereas on culture media luxuriant growth usually develops in which a variety of spore forms appear (Fig. 11a). This growth and sporulation is, in certain species only, influenced by added growth factors such as thiamin[3]. It is only by study of the saprophytic phase of the ringworm fungi that it is possible to identify them. Dermatophytes will grow on hairs *in vitro* producing the same kind of structures as are obtained on culture media[3]. The lytic action of enzymes of many dermatophytes growing in this way leads to the formation of transverse wedges in the hair shafts (Fig. 12), it does not occur, however, in the case of *T. rubrum*[1]. This appearance differs from the longitudinal tunnelling of hairs which develops in



FIG. 12.—*Trichophyton mentagrophytes*. Hair invasion *in vitro* showing multiple transverse perforations produced by fungus seen growing on surface (Lactophenol blue, × 450.)



(a)



(b)

Fi [illegible]

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FIG 12.—*Trichophyton mentagrophytes*. Hair invasion *in vitro* showing multiple transverse perforations produced by fungus seen growing on surface. (Lactophenol blue, ×450.)

natural or experimental infections, a pattern probably determined by the intrafollicular position of the hair shaft and by chemotactic influences in the region of the hair bulb The pathogenesis of tinea infections is described more fully elsewhere[8].

SUBCUTANEOUS INFECTIONS

Certain fungi and actinomycetes have the ability to parasitize subcutaneous tissues when they are introduced by trauma (Table 4)

TABLE 4
FUNGI CAUSING SUBCUTANEOUS INFECTIONS

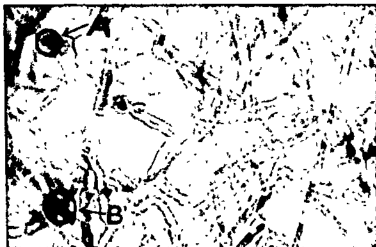
Fungus	*Disease*	*Classification*
Actinomyces israeli	Actinomycosis	B
Nocardia species	Actinomycetoma	B
Madurella species, etc	Maduromycetoma	F
Phialophora species	Chromoblastomycosis	F
Sporotrichum schencki	Sporotrichosis	D

B = Bacterium, F = Filamentous fungus, D = Dimorphic fungus

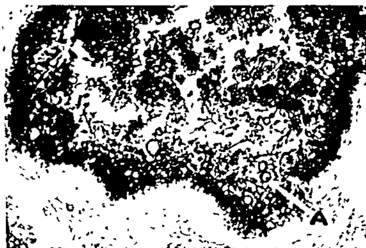
These organisms differ one from another in their ability to colonize at this site and in the tissue reaction and damage they elicit. In actinomycosis and actinomycetoma, small colonies or "granules" are formed which lead to the production of micro-abscesses in their vicinity In maduromycetoma similar lesions can be seen histologically around discrete colonies composed of true fungal mycelium (Fig. 13b), on culture media the same fungi grow profusely (Fig 13a)

In chromoblastomycosis, fungous growth in the tissues is much more restricted so that only small and dysgonic elements develop which proliferate slowly by a peculiar process of septation (Fig 14b) The presence of these small cells is, however, sufficient to give rise to micro-abscess formation and to granulomatous disease extending by histiocytic and lymphatic spread of the fungus. The tissue form of the fungus bears no similarity to its original saprophytic mycelial phase (Fig 14a)

Sporotrichosis, another example of a subcutaneous mycosis, comes closer to an infection in the bacterial sense The infecting mycelial fungus (Fig 15a) traumatically introduced into the tissues becomes converted to a yeast form (Fig 15b) In proliferating, the yeasts give rise to an intractable subacute inflammatory disease and are carried by lymphatics to form further subcutaneous granulomata at sites distant from the primary lesion



(a)

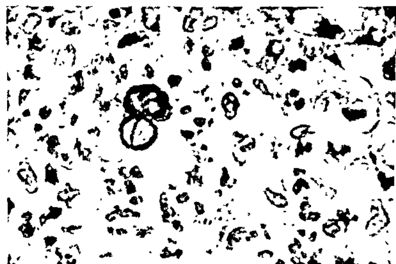


(b)

FIG 13 —*Madurella mycetomi* (a) Saprophytic phase Coarse mycelium bearing lateral spores truncated by septa (A) and thick-walled chlamydospores (B) (Lactophenol blue, ×900) (b) Parasitic phase Fungal colony in subcutaneous tissues in mycetoma consisting of coarse mycelium and thick-walled cells (chlamydospores) (A) Colony embedded in brown matrix secreted by the fungus forming a dark coloured "grain" (Periodic acid Schiff, ×250)



(a)

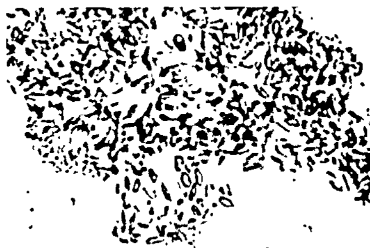


(b)

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(a)

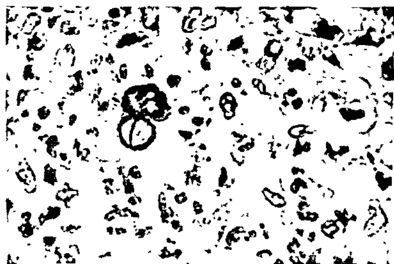


(b)

FIG 15 —*Sporotrichum schenckı* (a) Saprophytic phase Mycelium and *sporing structures (Lactophenol blue, ×900) (b) Parasitic phase Yeast* cells mostly oval in shape proliferating in pus from sporotrichotic lesion (×800)

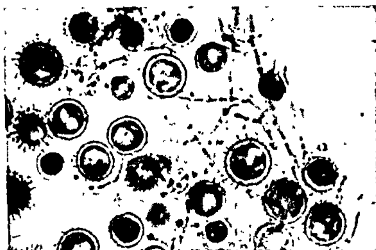


(a)

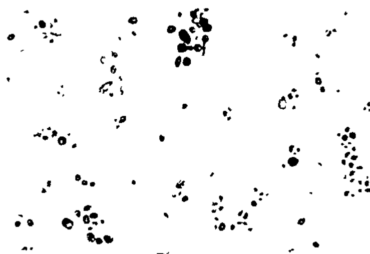


(b)

FIG. 14 [illegible] (a) Saproph[illegible] [illegible]



(a)



(b)

FIG 16 —*Histoplasma capsulatum* (a) Saprophytic phase Mycelium and thick-walled tuberculate spores from culture grown at 22° C (Lactophenol blue, ×800) (b) Parasitic phase Minute yeasts from culture grown at 37° C (Lactophenol blue, ×420)

PULMONARY AND SYSTEMIC INFECTIONS

The final group of fungi to be considered show well-developed pathogenic properties, the majority capable of producing disseminating diseases (Table 5)

Endogenous infection.—Infection may be endogenous in origin as in actinomycosis Here *A. israeli* departs from its usual role as a harmless commensal of the oropharynx or alimentary tract and becomes invasive in a new habitat *C. albicans* is similar in this respect but, though its invasive potentialities are not infrequently seen in the respiratory or alimentary mucosae at autopsy, it only rarely produces systemic infection, and pneumonitis due to *C. albicans* is probably non-existent.

Infection by dust inhalation.—Distinct from these endogenous infections are the pulmonary and systemic diseases acquired by inhalation of fungus-infected dust They are usually benign, but are fatal in the rare cases where fungus cells are disseminated to brain, skeletal structures, skin and elsewhere The invading fungus in each case is unicellular, this being its natural form in torulosis (Fig 2) and its parasitic form by adaptation in histoplasmosis (Fig 16b), coccidioidomycosis (Fig 17b), and blastomycosis (Fig 4b)

TABLE 5

FUNGI CAUSING PULMONARY INFECTIONS

Fungus	*Disease*	*Classification*
Endogenous		
Actinomyces israeli	Actinomycosis	B
Candida albicans	Moniliasis	Y-L
Exogenous		
Primary pathogens		
Cryptococcus neoformans	Torulosis	Y
Histoplasma capsulatum	Histoplasmosis	D
Coccidioides immitis	Coccidioidomycosis	D
Blastomyces dermatitidis	Blastomycosis	D
Secondary invaders		
Aspergillus fumigatus	Aspergillosis	F
Rhizopus species	Mucormycosis	F

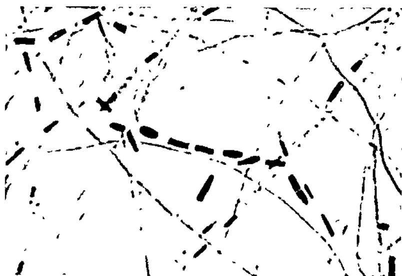
B=Bacterium, Y=Yeast, Y-L=Yeast-like fungus, F=Filamentous fungus, D=Dimorphic fungus

By contrast, in aspergillosis and mucormycosis the causative fungi are not dimorphic and, in consequence, produce only localized disease rarely metastasizing.

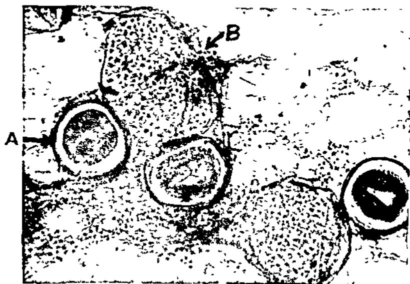
It is not known how frequently *C. neoformans* is inhaled in dust, nor how often it produces minimal pulmonary lesions Since laboratory-acquired infections appear to be unknown, it would seem that this yeast has only low pathogenicity for man. In susceptible subjects it gives rise to pulmonary granulomata which may progress to form widespread areas of pneumonitis Where proliferation of yeasts is in advance of the tissue reaction a tumour-like mass (toruloma), consisting almost entirely of yeasts and capsular material, is produced (Fig. 18) The commonest sites for distant lesions are the meninges and brain and, in these, as well as in pulmonary



FIG 18 —Toruloma of lung Resected specimen (left lower lobe) from male, aged 55 years, who had not travelled outside Britain (*B*) *courtesy of Mr W P Cleland*)



(a)



(b)

Fi [illegible]

It is not known how frequently *C. neoformans* is inhaled in dust, nor how often it produces minimal pulmonary lesions. Since laboratory-acquired infections appear to be unknown, it would seem that this yeast has only low pathogenicity for man In susceptible subjects it gives rise to pulmonary granulomata which may progress to form widespread areas of pneumonitis. Where proliferation of yeasts is in advance of the tissue reaction a tumour-like mass (toruloma), consisting almost entirely of yeasts and capsular material, is produced (Fig 18) The commonest sites for distant lesions are the meninges and brain and, in these, as well as in pulmonary



FIG 18—Toruloma of lung Resected specimen (left lower lobe) from male, aged 55 years, who had not travelled outside Britain (*By courtesy of Mr W P Cleland*)

lesions, the similarity of the parasitic and saprophytic forms of the fungus is seen. There is frequently comparatively little reaction in tissues infected by this yeast, which may in some way be connected with the presence of its capsule On the other hand, it is possible that the pathogenicity of *C. neoformans* may be attributable to its ability to form a capsule.

In histoplasmosis, the infective spores inhaled from *Histoplasma capsulatum* mycelium have a typical tuberculate appearance (Fig. 16a); these then undergo a remarkable transformation into minute yeasts on reaching the pulmonary tissues (Fig *16b*) The granulomatous lesions which they invoke usually heal spontaneously, but on the rare occasions when the fungus spreads the outcome is often fatal; disseminated disease frequently takes the form of a reticulo-endotheliosis In coccidioidomycosis, thick-walled arthrospores, or chlamydospores (Fig 17a), constitute the infective elements, and in North American blastomycosis spherical spores borne singly are responsible (Fig. 4a) The corresponding tissue forms of the fungi *are respectively spherical cells (sporangia), dividing internally* to produce endospores (Fig 17b), and thick-walled yeast cells (Fig 4b). The pulmonary pathology in these three diseases is somewhat similar and has been mistaken for tuberculosis Caseation and calcification are features particularly of coccidioidomycosis and histoplasmosis respectively The diseases due to dimorphic fungi cannot be diagnosed histologically with any certainty unless the causative organisms are demonstrated in the infected tissues

Aspergillus fumigatus (Fig 1) does not appear to produce primary aspergillosis in human subjects of the kind which occurs in birds It may, however, invade and sporulate within the lumena of lung cavities and proliferate to form colonial masses sometimes reaching large dimensions (aspergillus mycetoma) (Fig 19) Similar proliferation may occur within bronchi distal to stenoses due, for example, to sarcoidosis or carcinoma, it may also take place in zones of bronchiectasis *A fumigatus* will colonize lung tissues damaged by bacterial infection, infarction, asbestosis and other processes It is believed that, as is the case for ringworm fungi in relation to viable skin structures, the signs and symptoms of aspergillosis result from the effects of diffusable fungous products in the lung

Rhizopus species (Fig 20) are responsible for mucormycosis, a disease of the antra, orbits, or lungs, sometimes disseminating to the brain[2]. It tends to occur in subjects debilitated by diabetes and leukaemia, and in patients receiving cortisone, antileukaemic drugs and certain antibiotics

In aspergillosis and mucormycosis the causative fungi are present



Fig 19 [illegible]



Fig 20 —*Rhizopus* species Mycelium with characteristic rhizoids (A) and sporing structure (sporangia) (B) (Lactophenol blue, ×214)

in the tissues as mycelium, occasionally producing sporing structures in air-containing spaces such as cavities

CONCLUSION

The pathogenic fungi comprise a small number of species which vary in behaviour between being little more than superficial skin saprophytes and active tissue parasites. Though their morphological characteristics may, to a certain extent, be equated with such differences, relatively little is known of the chemical and biological factors which render them pathogenic for man.

ACKNOWLEDGEMENTS

Acknowledgements are made to Mr R J Lunnon, Institute of Dermatology, for the photomicrographs, and to Mr. D. Kemp, Institute of Diseases of the Chest, for Figs 18 and 19.

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HISTOPATHOLOGICAL OBSERVATIONS

SOME CASES OF FUNGAL INFECTION SEEN IN GREAT BRITAIN

W. St. C. SYMMERS

The histopathological features of various deep-seated fungal infections will be considered under four headings: tissue reactions; aids to the histological detection and identification of fungal infections; simulation of fungi by other structures in tissues; the comparative morphology of some non-myceliate forms of fungi in tissue sections.

TISSUE REACTIONS IN FUNGAL INFECTIONS

The morphological manifestations of the response of the tissues to infection by fungi range from the practically complete absence of any reaction to the severest of inflammatory changes To some [illegible]

is no variety of tissue reactions which is in itself pathognomonic of infection by any particular fungus, and, conversely, the same organism may evoke different reactions in different circumstances The reasons for these variations in response are still largely obscure

The types of reaction listed in the following paragraphs are mentioned as a reminder of the variety of histological changes which may be seen, listing them in this way is certainly not intended to suggest that they necessarily have any specific aetiological or diagnostic significance, but merely that fungal infections may be manifested by various tissue reactions and, therefore, that the possibility of this cause must not be overlooked when interpreting such findings, particularly in biopsy material

No reactions.—Absence of any detectable reaction is unusual There may be no reaction around colonies of *Cryptococcus neoformans*, particularly in the brain (Fig 21) Similarly, although there is thrombosis of infected blood vessels in cases of mucormycosis, and leucocytic accumulation may be seen around the mycelium within the thrombus, there is sometimes no reaction around the mycelial threads which have grown into the tissues around the vessels (Fig 22) This lack of response does not merely reflect



FIG 21 —Torulosis Colonies of *Cryptococcus neoformans* in brain, no tissue reaction present (Biopsy, haematoxylin-eosin, × 105)



FIG 22 —Mucormycosis Mycelium of *Rhizopus* species in thrombosed arteriole and surrounding brain tissue, little or no tissue reaction present (Haematoxylin-eosin, × 400)

growth of the organism after death of the host tissue, for it may be seen in viable tissues in freshly fixed biopsy material.

Intracellular parasitism.—Parasitization of macrophages is the most characteristic feature of the infected tissues in cases of histoplasmosis Simple histiocytosis is characteristic of infection by *Histoplasma capsulatum* (Figs. 23 and 51) In cases of infection by the larger form of *Histoplasma*, which is often designated as a distinct species (*Histoplasma duboisi*), the parasitized cells are multinucleated giant cells of the type seen in banal foreign-body granulomas (Fig 52) In either type of histoplasmosis the number

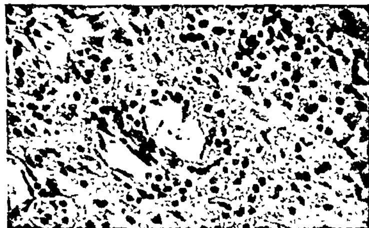


Fig. 23 [illegible]

of cells affected and the number of organisms in them vary greatly, a long search maybe necessary before a single organism is found, or field after field may be completely filled by heavily parasitized cells

Chronic non-specific, non-suppurative inflammatory reaction.—The chronic non-specific inflammatory reaction characterized by the accumulation of "small round cells", that is, lymphocytes and plasma cells, without suppuration or tuberculoid reaction, and tending to lead to fibrosis, is unusual in fungal infections other than rhinosporidiosis, of which it is characteristic Sometimes this sort of

reaction is all that is seen in the tissues around colonies of *Cryptococcus neoformans*, particularly in the meninges and around the so-called cerebral "torulomas" (Fig 24), and in some pulmonary lesions

Acute suppurative reaction.—Acute abscess formation is rare in fungal infections. Such lesions occur in some fulminating infections, such as a case of acute septicaemic torulosis and in the metastatic lesions in a case of acute monilial endocarditis in a drug addict who had been taking cocaine intravenously and whose supply of the drug was heavily contaminated by *Candida albicans*

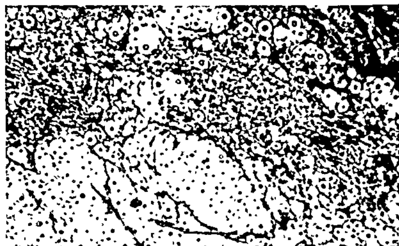


Fig 24 —Torulosis Margin of meningo-cerebral "toruloma", showing chronic non-specific inflammatory reaction (Haematoxylin-eosin, × 105)

Chronic suppurative reaction.—Chronic suppuration, with pus-filled sinuses surrounded by organizing granulation tissue devoid of specific features, is the typical tissue reaction in actinomycosis (Fig 25) and nocardiosis, and in the various forms of maduromycosis When such lesions are found in biopsy material it may be necessary to examine step sections through many blocks of tissue before colonies of the causative organism are seen, sometimes only sparse mycelial threads are to be found and colonies as such may be lacking. It is worth noting, too, that colonies of *Nocardia* in the tissues may be difficult to distinguish morphologically from *Actinomyces* in haematoxylin-eosin preparations, and that *Nocardia* may lose its acid-fast staining characteristic during processing of the tissues for histological examination



FIG 25.—Naturally-occurring actinomycosis in a ferret Colony of *Actinomyces* in pus in sinus lined by granulation tissue (Haematoxylin-eosin, ×150)



FIG 26.—Maduromycotic granuloma of the hand Darkly stained colony of *Allescheria boydi* in pus-filled sinus, with many large pale histiocytes in the pus and in the surrounding granulation tissue (Haematoxylin-eosin, ×170)

Chronic suppuration with histiocytosis.—Intermediate between the simple chronic suppurative reaction and a suppurating form of tuberculoid granuloma which will be described in the next paragraph is a variety of tissue response in which large histiocytes are a major constituent of the cellular reaction in the tissues around the pus-filled sinuses of some cases of maduromycosis (Fig. 26).

Suppurating tuberculoid granulomatous reaction.—This suppurating tuberculoid reaction (Figs. 27 and 28) is particularly important because it has considerable diagnostic value. The characteristic



FIG 27 —Sporotrichosis *Sporotrichum schenckii* was cultivated from this tissue Tubercle-like aggregates of epithelioid histiocytes and multinucleated giant cells, with extensive suppuration. (Haematoxylin-eosin, ×90)

"unit-lesion" of this type of reaction is the "suppurating tubercle" ("suppurating pseudotubercle" is a more accurate term), consisting of a rounded aggregate of epithelioid histiocytes with a central micro-abscess Whenever this type of lesion is found unexpectedly the possibility of a fungal infection must always be considered along with the other types of infection which may cause the reaction, such as viral infections, including lymphogranuloma inguinale and cat-scratch fever, and certain unusual bacterial infections, for example, tularaemia and *Pasteurella septica* infection

In the severer forms of this reaction, whether due to fungal, viral,

which

een in

septic

infection. The fungal infections in which it occurs include sporotrichosis, North American blastomycosis, South American blastomycosis, and coccidioidomycosis, and often the organism concerned is to be found at the middle of the purulent focus

Tuberculoid granulomatous reaction.—This is the most usual form of reaction to infection by the fungi which occur in the tissues in a yeast-like form; it is the typical response in the North and South American types of blastomycosis, and in chromoblastomycosis, coccidioidomycosis, and the so-called African (*Histoplasma duboisi*)

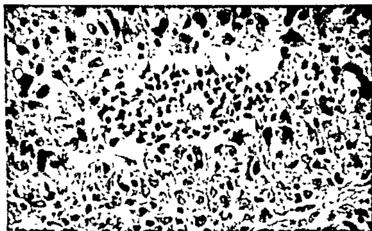


FIG 28 —Section from same specimen as Fig 27 Micro-abscess at centre of aggregate of epithelioid histiocytes ('suppurating pseudotubercle'), sporotrichotic "asteroid" in pus (Haematoxylin-eosin, ×400)

type of histoplasmosis Tuberculoid granulomas are also seen in many cases of torulosis (Fig 29)

Essentially, the reaction consists of focal aggregates of epithelioid histiocytes, with occasional multinucleated giant cells among them The general appearance may be similar to the classical tubercle, although usually without caseation, but commonly the tuberculoid reaction in the fungal infections is neither so well circumscribed nor so uniformly well developed as the usual "unit-lesion" of banal tuberculosis or of for instance, sarcoidosis Caseation appears to be unusual in fungal granulomas, it occurs most characteristically *in the tuberculoma-like, encapsulated lesions which are sometimes* found, especially in the lungs, as a solitary manifestation of coccidioidomycosis or histoplasmosis or, more rarely, torulosis In such

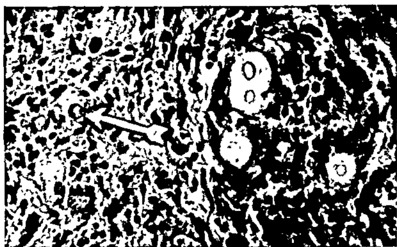


FIG 29 —Torulosis Organizing tuberculoid granuloma with multinucleated giant cells containing typical cryptococci An unencapsulated cryptococcus is also seen (arrowed) (Haematoxylin-eosin, ×400)



FIG 30 —Maduromycosis Foreign body giant cells around dead material (inspissated colony of *Allescheria boydi*) (Haematoxylin-eosin, ×400)

instances, the lesions may possibly be mistaken for old Ghon foci or other tuberculous lesions, or even for gummas, unless fungi are specifically sought. Caseation may, however, occur also in some more active fungal infections; it may develop, for instance, in miliary pneumonic lesions caused by *Blastomyces dermatitidis*.

Foreign-body reaction.—Occasionally, dead fungal material may become inspissated and evoke a typical foreign-body reaction: thus, in a biopsy specimen (Fig 30) from a mass in a foot, brownish-yellow amorphous foreign material surrounded by foreign-body giant cells caused much speculation until it was seen to contain curious channels and little vesicle-like spaces, suggesting the outlines of a spore-bearing mycelium. Further investigation led to isolation of *Allescheria boydi*, confirming the presence of maduromycosis

AIDS TO THE HISTOLOGICAL DETECTION AND IDENTIFICATION OF FUNGAL INFECTIONS

Often, the presence of fungi in the tissues is readily apparent in the first sections examined, and special methods of investigation then provide confirmatory evidence only. For obvious reasons, if the possibility of a fungal infection has been raised and biopsy is contemplated, steps should always be taken to try to isolate the organism by culture; reliance should not be placed wholly on histological study, invaluable though this can be

The histological methods which can profitably be applied to the investigation of fungal infections are representative of the whole range of histological techniques. They may be discussed briefly under the headings of optical methods and staining

Optical methods.—Some yeasts and yeast-like organisms may be readily demonstrated in polarized light because of bi-refringent effects which they produce (Figs 31 and 32), and this characteristic is sometimes invaluable in disclosing the presence of fungi in sections Such organisms are among the unsuspected structures which can easily escape observation unless the microscopist makes it a regular practice to keep a Polaroid filter constantly in position between the light source and the object The amount of inherent polarization ordinarily present in the prism system of binocular microscopes is then quite sufficient to show up bi-refringent material in the preparation examined, even against the ordinary bright field This simple procedure can be of great value in diagnostic histology, particularly in interpreting the significance of granulomatous lesions

The bi-refringence of fungi seems often to be induced by the procedures involved in processing tissues for sectioning, or in staining, thus, the organisms in frozen sections of formalin-fixed

material may show no bi-refringence while those in paraffin sections of the same specimen may be strikingly revealed in polarized light

Phase-contrast microscopy does not seem to have any special value in studying sections of mycotic lesions However, by using conventional objectives of low magnifying power with the substage phase rings appropriate to high-power phase objectives, a low-

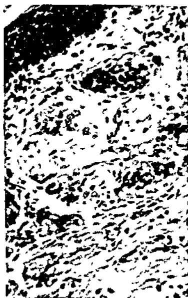


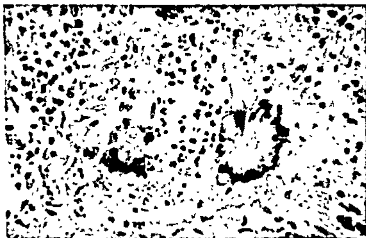
FIG 31 —Cutaneous histoplasmosis (*Histoplasma duboisi* infection) The pale fungi contrast with the darkly stained nuclei of the phagocytic giant cells (Haematoxylin-eosin, ×196)



FIG 32 —Same field as Fig 31 photographed in polarized light The white spots are yeast cells of *Histoplasma capsulatum*, only some of the organisms appear to be doubly refractile

magnification dark-ground effect is obtainable which may occasionally be helpful in revealing scanty organisms, as well as in studying the topography and development of the tissue reaction

***Staining.**—Although fungi in tissue sections are usually readily* detectable in haematoxylin-eosin preparations (Fig 33), there is no doubt that other stains often reveal them more strikingly, the periodic acid Schiff reaction, with haemalum as a counterstain, and various comparable procedures such as the Gridley stain, are among the most useful



[illegible]



FIG. 31 [illegible] (× 200)

Silver methods, such as the Foot or Robb-Smith techniques for silvering reticulin fibres, show up some fungi very well, including most of the yeast-like organisms and also *Aspergillus* (Fig 34) Gram's stain, on the other hand, is more valuable in disclosing some of the finer details of the inner structure of the organisms than as a selective stain (Fig 35). Mucicarmine is often used to stain the capsular mucopolysaccharide substance of cryptococci. At least some strains of *Blastomyces dermatitidis* and *Blastomyces brasiliensis*



Fig 35.—Torulosis Internal structure in yeasts demonstrated by Gram's stain (× 400)

are also mucicarminophilic, their cell surface having a distinct affinity for the stain This affinity is most readily demonstrated and most intense in preparations which have been exposed as little as possible to watery solutions, the mucicarminophile material being evidently fairly readily extracted by water

A valuable "battery" of stains for demonstrating fungi consists of Mayer's haemalum and eosin, periodic acid Schiff and Mayer's haemalum, Southgate's mucicarmine and Mayer's haemalum, and Robb-Smith's method for silvering reticulin

Other important factors —It is perhaps appropriate to mention two other obvious but most important factors which help towards the histological recognition of fungal infections these are curiosity and patience

Curiosity is required to encourage adequate investigation of unexplained inflammatory processes, and to inquire into the reasons for

unexpected histological appearances. A case of histoplasmosis was, for example, recognized because a pathologist was curious to know the reason for the profusion of multinucleated giant cells in the tuberculoid granulomas in a fresh biopsy specimen in contrast with the absence of giant cells at biopsy some years earlier when the patient had been shown to have sarcoidosis.

Patience is necessary where examinations of many hundreds of sections for the presence of recognizable organisms has to be carried



FIG 36 —Chromoblastomycosis (*Phialophora* (*Hormodendrum*) *pedrosoi* infection) In this unstained section the fungal bodies are clearly seen because of their natural yellow-brown pigmentation (×850) (*See also* Fig 55)

out, as in looking for the rare "asteroid" of sporotrichosis, and as in some cases of other fungal infections in which the organisms may occasionally be very scanty

SIMULATION OF FUNGI BY OTHER STRUCTURES

Many structures can simulate fungi, but care and a little experience should prevent confusion The following are by no means the only simulants, but they are noted to illustrate the sort of problems which occur.

(*a*) Cross-section of a nerve in a granulomatous lesion mistaken for the endosporulating spherule stage of *Coccidioides* (Figs 37 and 46) (*b*) Groups of nerve fibres mistaken for colonies of cryptococci because the axons, each with its surrounding clear myelin sheath, mimicked encapsulated yeasts (Fig 38) (*c*) Calcific spherules in tuberculous lesions (Fig 39), or tumours (Fig 40) mistaken for yeasts

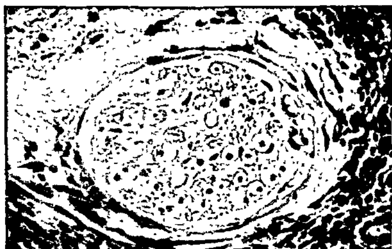


FIG 37 —Bundle of nerve fibres in a chronically inflamed tissue This nerve was mistaken for the endosporulating spherule of *Coccidioides* (Haematoxylin-eosin, ×400) (*See also* Fig 46)



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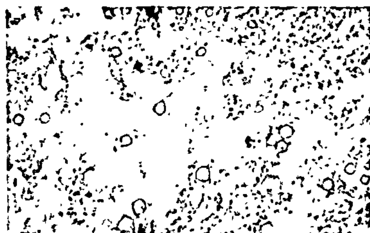


Fig 39 [illegible]

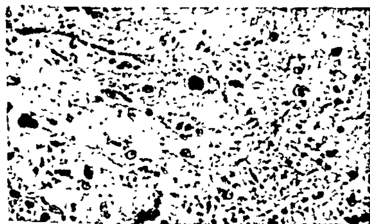


Fig 40 — Calcifying bodies simulating yeasts in an oligodendroglioma The calcifying mycelium-like structures are probably nerve fibres (Haematoxylin-eosin, × 400)

macrophages in the germinal centres of lymphoid follicles in reactive hyperplasia, mistaken for *Histoplasma* (Fig. 42) (*e*) Intracellular globules of mucin in scirrhous mucigenic carcinomas (Figs. 43, 44, and 45) mistaken for phagocytosed cryptococci (*f*) Cytoplasmic vacuoles of all sorts, particularly in the epithelioid histiocytes of tuberculoid granulomas, mistaken for yeasts What

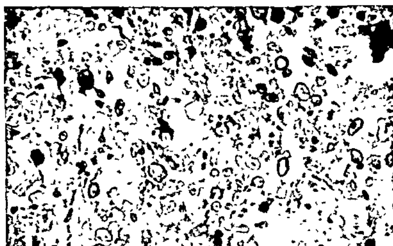
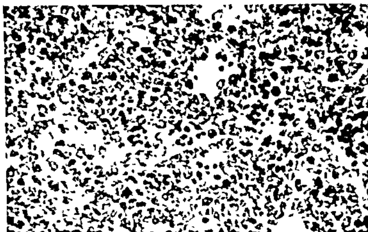


FIG 41 —Torulosis Calcifying cryptococci in cerebellar granuloma, *Cryptococcus neoformans* was isolated from this lesion (Haematoxylin-eosin; ×400)

is important is not just that fungi can be simulated by other structures but that as much care must be taken before dismissing the fungal nature of a debatable structure as must be taken when making the histological diagnosis of a mycosis

COMPARATIVE MORPHOLOGY OF SOME NON-MYCELIATE FUNGI AS SEEN IN SECTIONS

Photographs of various organisms at the same high magnification (Figs. 46–56) illustrate that neither size nor general appearance is enough to identify these fungi specifically in every case However,



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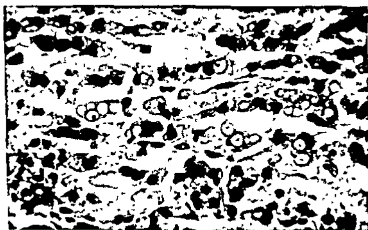


FIG 43 —Intracellular bodies simulating yeasts in a swelling of an eyelid of as yet unidentified nature (Haematoxylin-eosin, ×400) (*See also* Fig 44) (*By courtesy of Professor D M Pryce*)

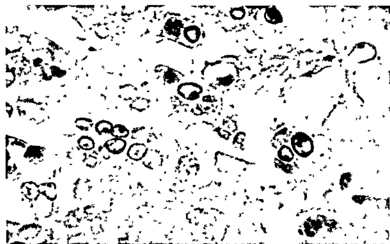


FIG 44 —Same specimen as Fig 43 The intracellular bodies may be mucicarminophile fungi in macrophages or droplets of mucin in tumour cells (Southgate's mucicarmine and Mayer's haemalum, × 600) (*See also* Fig 45)

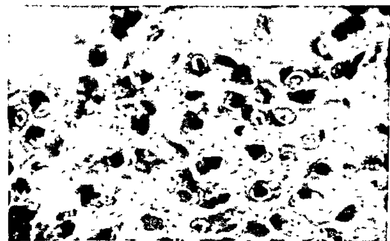


FIG 45 —Preparation of a scirrhous mucigenic carcinoma for comparison with Fig 44 Some of the mucinous vacuoles in the tumour cells have a mucicarminophile rim and contain an eccentric mucicarminophile globule this appearance is also seen in Fig 44, but *see also* Figs 35 and 52 (Southgate's mucicarmine and Mayer's haemalum, × 600)

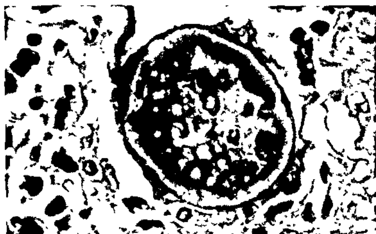


FIG 46 —Coccidioidomycosis Endosporulating spherule of *Coccidioides immitis* in a lymph node thought clinically to be tuberculous, from a British nurse who had lived in California (Haematoxylin-eosin, × 850)



FIG 47 —Same specimen as Fig 46 Groups of maturing spherules of *Coccidioides immitis* in macrophages (Haematoxylin-eosin, × 850)

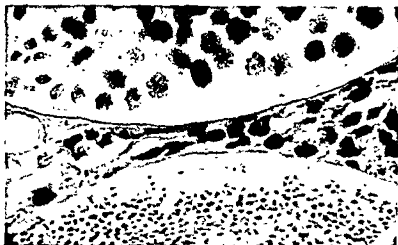


FIG 48 —Rhinosporidiosis in an Indian student working in Britain Parts of two sporangia of *Rhinosporidium seeberi* containing spores of different sizes (Haematoxylin-eosin, × 850)



FIG 49 —Torulosis Typical yeasts of the classical large-cell type of *Cryptococcus neoformans* with broad capsular zone (Haematoxylin-eosin, × 850)

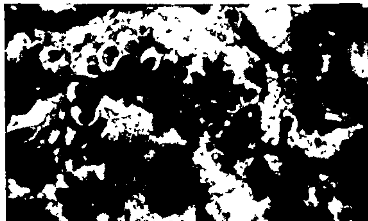


FIG 50 —Torulosis Small-cell type of *Cryptococcus neoformans* with narrow capsular zone in giant cells of a tuberculoid reaction in a skin lesion (Haematoxylin-eosin; × 850)

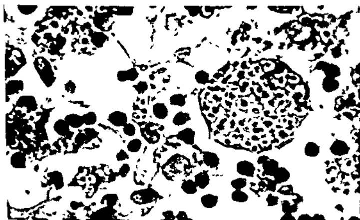


FIG 51 —Histoplasmosis Macrophages in sinus of a lymph node heavily parasitized by classical small-cell type of *Histoplasma capsulatum* (Periodic acid Schiff reaction, Mayer s haemalum, × 850)

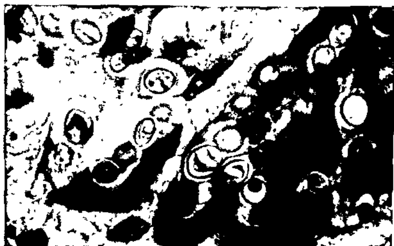


FIG 52 —"African" type of histoplasmosis Large-cell type of *Histoplasma* (*H duboisi*) in multinucleated giant cells of dermal granuloma (Haematoxylin-eosin, × 850)



FIG 53 —South American blastomycosis Yeasts of *Blastomyces brasiliensis* within a multinucleated giant cell in a subcutaneous granuloma from a British engineer who had worked in Brazil (Periodic acid Schiff reaction and haemalum, × 850)

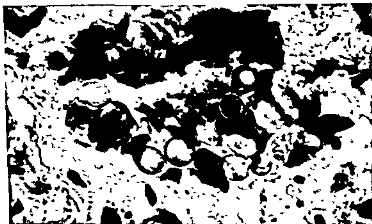
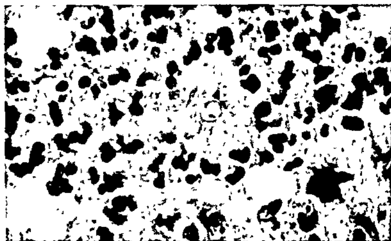


FIG 54—North American blastomycosis Yeasts of *Blastomyces dermatitidis* within a multinucleated giant cell in a dermal nodule from an American resident in London (Periodic acid Schiff and haemalum, ×850)



FIG 55 —Chromoblastomycosis Group of organisms (*Phialophora* (*Hormodendrum*) *pedrosoi*) at one pole of a multinucleated giant cell (Haematoxylin-eosin, ×850)



[illegible]

blastomycoses and histoplasmosis, and in cases of coccidioidomycosis when the endosporulating spherule stage is found in the tissues. Whenever cultivation is possible, however, isolation of the organism is necessary for definitive diagnosis, and for pathogenicity studies. That even seemingly pathognomonic pictures may prove to be equivocal is shown by the recent observations of Capponi, Sureau and Segretain It could once be confidently said that a macrophage filled with small, double-contoured, yeast-like bodies was typical of histoplasmosis This picture no longer has the same absolute diagnostic import since Capponi and his colleagues observed an identical appearance in animals infected with a pathogenic species of *Penicillium, Penicillium marneffei,* isolated from a naturally occurring infection in the Vietnamese bamboo-rat This observation reflects the care necessary to ensure by all possible means the correct and complete diagnosis of fungal infections.

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TINEA INFECTIONS

PATHOGENESIS

R VANBREUSEGHEM

ALTHOUGH the word "dermatophyte" has been used in the past to cover every type of the vegetable micro-organisms living on the surface of the skin, bacteria as well as fungi, it is now usually restricted to the pathogenic fungi which produce ringworm in man and animals They constitute a group of fungi which have for morphological reasons been related to the Ascomycetes, but no real proof of their relation with this class of fungi has yet been found Until such proof exists, they must be considered as *Fungi imperfecti* in that they do not exhibit any sexual form of reproduction Although from the microscopical point of view they have a rather characteristic morphology, they distinguish themselves from the other fungi by their ability to produce tinea infections and by their power to attack and digest keratin *in vitro* as well as *in vivo* As parasitic agents they manifest a true keratinophilia, this fact being demonstrated long ago by Sabouraud[23] and more recently by Kligman[17, 18]

For 100 years, the dermatophytes have been considered as strict parasites living on the skin, hair, nails, feathers, and horns of various animals, although their ability to grow on widely differing types of culture media was well recognized Although they could be true parasites, they were also able to live outside of the body as saprophytes This interchange between saprophytism and parasitism is a characteristic of the dimorphic pathogenic fungi, and the question arises as to whether it applies also to the dermatophytes

RECENT FINDINGS

In the last few years it has been shown that, like other pathogenic fungi, some dermatophytes live normally in the soil This has been demonstrated in the case of 3 species in 3 different genera *Keratinomyces ajelloi*, a non-pathogenic dermatophyte, *Microsporum gypseum*, and *Trichophyton mentagrophytes* From recent studies made by Georg[12] it appears that, although strains of *K ajelloi* as described by Vanbreuseghem[26] are mostly non-pathogenic, certain strains are probably able to produce ringworm in some animals

This fungus, and also *Mic. gypseum*, has been isolated repeatedly from the soil in various parts of the world[1, 7, 9] *T. mentagrophytes* has been isolated in South Africa from soil[20] and also from the air of a cave[21].

Dermatophytes, like many other fungi, may be cultured in the laboratory on sterile soil[30]. The real proof that at least one of them grows naturally in the soil has been provided by Gordon[13] in the case of *Mic gypseum*, for this author was able to demonstrate macroconidia typical of this fungus in the soil itself

T. mentagrophytes, *T. rubrum*, and *Epidermophyton floccosum* have been isolated from the surroundings of animals, and from the floors of swimming pools[2, 10]. As Gentles has pointed out, it should not be assumed that these dermatophytes live on floor structures, for they may merely persist in desquamated keratin fragments. Similarly, dermatophytes growing in a natural state on animal droppings do not have the same significance as dermatophytes growing within the soil[29]

Many more attempts have been made to isolate dermatophytes from the soil than is reflected in the literature, yet successes have been few. It would seem unlikely, however, that only a few species of dermatophytes are able to thrive away from the human or animal environment. Immense quantities of dermatophyte spores fall out of the integuments of infected humans and animals and become, for short or long periods, a living part of the environment It cannot be assumed that the spores develop further in such surroundings, for it could be that they are destroyed by antibiotics[14], or by other agents or conditions But dermatophytes must exist in our environment if only for short periods and our inability to isolate them merely indicates the present imperfection of our methods

Means of infection.—It was postulated some years ago that "the soil might constitute the great well of human and animal dermatophytosis epidemics"[27] While tinea infections are usually acquired by transmission of infection from man to man, from animals to animals, and from animals to man or the reverse, these are certainly not the only ways[29, 33] Thus, the observations by Gentles and Holmes[11] favour the view that infection is readily acquired in bathing places

The dermatophytes are very specialized parasites in being limited to keratinaceous structures by virtue of their keratinolytic power and their keratinophilia Some exceptions have been noted, as for example in rare cases where *T schoenleini* has invaded the deeper tissues of the skin, lymph nodes, and even the blood stream[16] When dermatophytes are introduced into the bodies of experimental

animals by ways other than through the skin, they manifest a very selective epidermotropism This fact has been illustrated experimentally by Brocq-Rousseu, Urbain and Barotte[4]. These authors were able to produce ringworm in guinea-pigs by administering an emulsion of spores of *T. mentagrophytes* or *T. equinum* by intravenous, intraperitoneal, subcutaneous, and oral routes if, at the same time, they slightly traumatized the skin of these animals The delay in the development of the skin lesions by this routine, compared with that after direct inoculation in the skin, was only 2 or 3 days

Lurie and Way[21] have demonstrated that spores of dermatophytes may be introduced into animals by inhalation and remain alive *in vivo* for at least 8 weeks *Ep floccosum* has been isolated from sputum[31] but contamination of the sputum sample had most likely occurred after expectoration.

LaTouche[19] has studied the transmission of ringworm by contact and Kligman[17] has indicated the need for slight trauma in establishing experimental ringworm infections The contagiousness of tinea pedis and of onychomycosis is much debated Maceration of the feet and repeated exposure in places where infected skin scales are present appear to be the commonest aetiological factors; trauma too may be important. Degrees of contagiousness are difficult to assess and it is also difficult to ascertain the time of onset of an infection and the reasons why clinical symptoms become manifest The reason for the apparent immunity to ringworm infection of some nails of a hand compared with the vulnerability of others is unknown. It is possible that trauma to some particular part of the nail plate is an important prerequisite before infection occurs[32]. In contrast, tinea capitis is usually acquired by close contact, and the same is usually true for tinea corporis. There remain the possibilities that transmission of infection by inhalation and by ingestion may occur

In spite of numerous studies upon the keratinolytic properties and keratinophilia of dermatophytes[3, 5, 6, 22, 24, 25], precise knowledge of the mode of keratinolytic action is scanty Little is known either of the mechanism of inflammatory reactions in ringworm or of the factors involved in immunity, allergy, or cure of these diseases On the other hand, the morphology of tissue reactions of all types has been described with a high degree of precision

Living conditions and susceptibility.—The susceptibility to ringworm of different peoples living under different conditions would repay careful study Under the conditions most favourable for transmission of infection in civilized countries, tinea capitis does not spread to more than 5 per cent of children Boys are more often

affected by tinea capitis than girls, and the disease tends to cease at puberty in some types of infection though not in others; tinea capitis caused by *T. violaceum* persists in female adults more often than in males[15]. These are some of the unsolved problems met with in temperate countries. Personal observations[28] on native children of Central Africa have demonstrated a very large variation in the incidence of dermatophyte infections among them Thus, 5 per cent of some groups of children suffered from tinea capitis, while up to 40 per cent of others were involved. These differences could not be explained by standards of housing, education, or hygiene; nor could they be attributed to variations in virulence of the dermatophyte strains concerned. Dietary factors could, however, have explained the difference, for the less affected children had [illegible]

be expected to be most affected by tinea capitis It has been shown in one study that nearly 95 per cent of kwashiorkor children suffered from tinea capitis It does not necessarily follow that protein deficiency is in itself the essential predisposing factor in this high incidence; avitaminosis, such as vitamin A deficiency, so often accompanying protein deficiency states, could also be important This might lead to deficiency in the quantity or quality of sebaceous gland secretion or to some alteration in the physical structure of hairs or skin which make for susceptibility to disease. It is of interest that work carried out in France by de Graciansky and his colleagues[8] suggested that a diet deficient in protein favours the development of *Candida albicans* infection in experimental animals

Conclusions.—A better understanding of the mode of transmission of ringworm would help to prevent spread of disease, a more complete knowledge of the physiology of pathogenic fungi would help towards the discovery of more efficient therapeutic agents, and a greater knowledge of natural and acquired immunity mechanisms in dermatophyte infections would help to explain much of the behaviour of these diseases

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TRICHOPHYTON RUBRUM INFECTION

C. D. CALNAN

IN company with many other European countries such as Germany, Holland and Switzerland, as well as the United States, we in Great Britain have experienced over the past decade a remarkable increase in the incidence of *Trichophyton rubrum* infection. It is likely that this infection has been endemic in China, Japan and the Far East[2, 4] for a long time, and that during the last 20 or 30 years it has spread progressively westwards over Asia and Europe, and eastwards to Australia and America In New York, according to Sulzberger[7], *T rubrum* infections are five times as common as those due to *T mentagrophytes*, and because of their intractability they are causing anxiety The disease was seen only sporadically before the second world war at St John's Hospital for Diseases of the Skin in London. Between 100 and 200 new patients infected with *T. rubrum* are now registered each year This increase is both real and apparent. Some of the real increase is due to immigrants and troops returning from overseas, but many new cases have never been abroad. Some of the increase is probably due to a raised index of suspicion in connexion with this particular fungus Apart from this, however, one knows that fungal infections in various countries do change their incidence over a period of years without any satisfactory explanation

EXCEPTIONS TO GENERAL CLINICAL PICTURE

From the work of Lewis and Hopper[3] and others in the United States the clinical picture typical of these infections was well known (Fig 57), but a number of exceptions to this general pattern occur The clinical features alone are not diagnostic of the infection for it is now agreed that more than one fungus species may produce the same signs

Feet.—Ordinary toe-web maceration may be caused by *T. rubrum* but the webs are usually considered to be spared In fact, the toe spaces show scaling more often than not, and fungus mycelium is present The dorsa of the feet and ankles are commonly involved, sometimes accompanied by an acute turgid papular erythema with little or no scaling, this may cause difficulty in diagnosis from erythema multiforme or chronic annular erythema Vesicular and

pustular lesions, sometimes of the acute type, may occur on the feet more like those associated with *T. mentagrophytes.* But, however acute the lesion, a genuine secondary trichophytide eruption on the hands does not appear to occur. Involvement of the toenails is extremely common though not invariably present

Hair.—Hair infection is being found more often, the fungus producing either an ectothrix or endothrix invasion It occurs in the coarse hair of the beard and scalp, occasionally causing kerion, as well as in lanugo hairs, especially on the legs and hands Frequently, however, this fungus spreads extensively over hairy parts of the skin

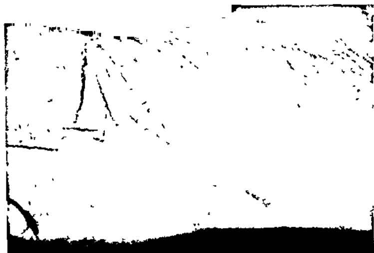


FIG 57.—*Trichophyton rubrum* infection Fine scaling of sole of foot (*By courtesy of Dr H Wallace*)

without any attempt to penetrate hair follicles On the legs follicular invasion which is confined mainly to women simulates erythema induratum or nodular vasculitis, or banal folliculitis Pustules on the legs do not always indicate hair infection, sometimes these do not represent follicular involvement and the roof of the pustule contains a mat of fungus similar to the pustules of ordinary ringworm of the feet The diagnosis will not usually be missed if the feet are examined, for the feet are rarely spared

Hands.—The hands may show 5 different types of lesions: hyperkeratotic palm and fingers (Fig 58), crescentic exfoliating scales, as occur on soles of feet, vesicular circumscribed patches, discrete red

papular and follicular scaly patches; and erythematous scaly sheet on dorsum.

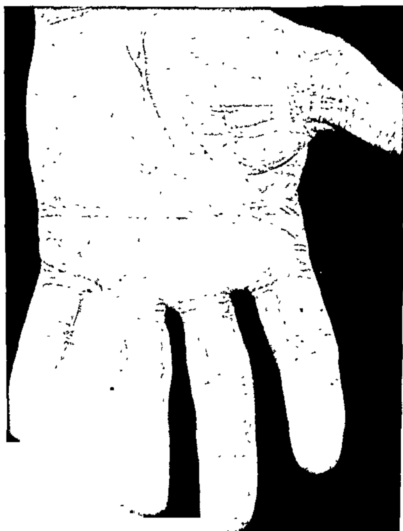


FIG 58 — *Trichophyton rubrum* infection Hyperkeratotic palm and fingers (*By courtesy of Dr R M B MacKenna*)

The hyperkeratotic palm type is the commonest and is unilateral in 50 per cent of cases, the vesicular eczema-like patches are the

most unusual Hand infections are readily misdiagnosed, especially in the absence of nail involvement Infections of the hand due to fungi other than *T. rubrum* are very rare in England, and finger-nail infections are almost invariably caused by this fungus

On the glabrous skin, *T. rubrum* may cause typical annular lesions of body ringworm, but other patterns appear to be more frequent Crural infections may be indistinguishable from those produced by *Epidermophyton floccosum*, but *T. rubrum* infection often spreads



FIG. 59 —*Trichophyton rubrum* infection Circinate lesion of glans penis (*By courtesy of Dr R Bettley*)

down the thighs and over the perineum to the buttocks Penile infections are very rare (Fig 59) Distribution tends to be asymmetrical Tinea cruris is almost unknown in women[1] but when it does occur *T rubrum* seems to be almost invariably responsible Any part of the trunk, head (Fig 60), or limbs may be affected There may be very large sheets of scaling with or without a well-defined marginated border, and sometimes there is lichenification suggestive of a diagnosis of neurodermatitis

A variant which is troublesome from the point of view of diagnosis is the raised turgid papular erythema without scaling It appears

as papules, plaques, crescents or rings After a few days this type of reaction may subside spontaneously leaving some scaling Alternatively, there may be large rings or plaques on the trunk or



FIG 60—*Trichophyton rubrum* infection Erythematous scaly lesion of ear (*By courtesy of Dr B Russell*)

elsewhere, with virtually no erythema and a minimum of scaling at the edges Such a lesion occurring in a patient presenting with pruritis may be extremely difficult to see and is readily overlooked

Mixed infections are not uncommon, *T mentagrophytes* and *Ep*

floccosum being the most common additional fungi present[5]. *Microsporum canis*, *T. tonsurans* and *T. violaceum* also occur together with *T. rubrum* In patients with clinical appearances of *T. rubrum* infection but from whom only other fungi were grown, the species concerned have been *Ep. floccosum* and *T. mentagrophytes* Nails were not involved in these patients and several, but not all, were cured with treatment.

Familial infections are not as rare as is sometimes supposed. Relatives are often reluctant to be examined and usually deny the existence of skin abnormalities even on their feet. An appreciable percentage of marital partners, however, have the infection, if only in the toe webs In the case of children and adolescents parental infection will almost always be found; infection has been seen in a baby of 10 months.

In 1946, Rothman in Chicago noted an increased glucose tolerance in 2 patients with *T. rubrum* infection and reported similar findings on 20 patients in 1953[6]. At St. John's Hospital for Diseases of the Skin, of 23 patients examined only 3 showed similar flat glucose tolerance curves.

Another apparent aetiological factor is acrocyanosis. Many of the patients with this disease have a perniotic circulation, especially those with hand involvement and nodular follicular infections on the legs. But chilblain circulation is a common disorder in Britain, and its relation to this disease is difficult to assess without statistical evidence.

The trichophytin test is not widely used in Britain A delayed positive reaction at 48 hours is associated with trichophytide reactions While most *T rubrum* infected cases give negative delayed trichophytide reactions, a small proportion give positive results, these exceptions did not have vesicular lesions, nor were they any less resistant to therapy The majority of cases show an immediate positive urticarial wheal reaction the reason for which is obscure; circulating antibodies have occasionally been demonstrated in such patients

Inoculation of the skin of *T rubrum* infected patients or of normal subjects with a culture of this fungus produces only an acute inflammatory lesion which easily resolves either spontaneously or with the aid of a fungistatic substance, it does not seem possible to produce in this way lesions which are typical clinically of *T rubrum* infection

TREATMENT

The challenge in this disease is its resistance to treatment. Body lesions, apart from the palms and soles, are often cured remarkably

as papules, plaques, crescents or rings. After a few days this type of reaction may subside spontaneously leaving some scaling. Alternatively, there may be large rings or plaques on the trunk or



FIG 60—*Trichophyton rubrum* infection Erythematous scaly lesion of ear (*By courtesy of Dr B Russell*)

elsewhere, with virtually no erythema and a minimum of scaling at the edges Such a lesion occurring in a patient presenting with pruritis may be extremely difficult to see and is readily overlooked.

Mixed infections are not uncommon, *T mentagrophytes* and *Ep*

EPIDEMIOLOGY OF *TRICHOPHYTON RUBRUM* INFECTION

MARY P. ENGLISH

THE problem of the epidemiology of *Trichophyton rubrum* infection is, by and large, the problem of all other dermatophyte infections of the glabrous skin, with certain additional special features

Dermatophytosis due to *T. rubrum* was undoubtedly originally a disease of the tropics. Sabouraud had never seen it when he wrote his classic *Les Teignes*[13] which appeared in 1910 In that year Castellani[4] and Bang[2] (the latter working in Sabouraud's laboratory) published, quite independently, descriptions of the fungus found in Ceylon, Mexico and Indo-China Sabouraud[14] a year later concluded that they were the same fungus, namely, *T rubrum* Sabouraud also recorded the fungus from India and Siam, so that it is probable that it was widespread in the tropics at that time.

Unfortunately, little is known about *T rubrum* in its native environment, even today. Clarke and Walker[5] in 1953 found that it was the most frequent agent of tinea of the glabrous skin in Nigeria, and Vanbreuseghem[17] reported 3 different strains of the fungus from the Belgian Congo Sanderson and Sloper[15] found it to be the cause of skin lesions in 7 out of 9 Asiatic soldiers in Malaya and Chakraborty (quoted by Maskin and others)[11] stated, though no evidence was given, that 80 per cent of dermatophytosis in India was due to this fungus

RISE IN INCIDENCE

The figures for the spread of *T rubrum* into Europe and North America since 1910 are striking In Canada in 1947, 23 3 per cent of all fungous infections were attributed to *T rubrum*[7] Maskin and his colleagues[11] showed that at the New York Clinic the incidence of the fungus has increased from 20 per cent to 80 per cent of fungous infections of the glabrous skin since 1935. In Europe a great increase has been recorded in Germany[10], Switzerland[3] and Holland[6] In Britain, Walker[18] isolated the fungus 97 times between 1946 and 1949, and over the last 3 years in Bristol skin clinics, *T rubrum* has been isolated more often than any other single fungus species[8] These figures, mostly obtained through clinics or medical practitioners, should not be taken to indicate that *T rubrum* is the chief

easily by clinical standards even when they have been present for many years, but the success rate falls considerably if repeatedly negative skin scrapings are demanded as the index of cure. *T. rubrum* infection of the palms and soles is equally resistant whether the nails are involved or not. The fungus is often surprisingly persistent and readily found in the skin during treatment with fungistatic agents Nail infection is at present virtually incurable, but individual infected finger-nails do sometimes return to normal as other nails become involved Though a nail may appear to have returned to normal it may yet contain fungus. Palm and sole infections are rarely cured, and examples of cure may, in fact, have been spontaneous remissions

No one fungicide is preferred to another, irrespective of its ability to inhibit *in vitro*. Surgical removal of nails has, in general, been abandoned for there is almost invariably an associated persistent infection on the adjacent skin from which re-infection may occur. The chemical removal of a nail appears to give no better results. Histological examination of a chronically infected nail using periodic acid Schiff technique for staining shows penetration of the fungus to the deepest crevices of the nail plate and illustrates the difficulties of treatment.

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first noticed while the patient was using swimming baths frequently, or taking an active part in sports. The remaining 8 had no idea where they might have contracted the disease

Women appeared to be just as susceptible as men, and children as adults, provided the opportunity for infection was there. In the great majority of cases the original infection was in the feet. Nail infections occurred in 78 per cent of all patients Eruptions elsewhere on the body appeared to be more often the result of auto-inoculation from the feet than cross-infection from another person

The evidence from this investigation shows conclusively that the *home is a likely place for cross-infection by T. rubrum, contrary* to the opinions of Sulzberger, and other American workers[16]. It also bears out the results of Baer and others[1] who showed that most individuals have a considerable resistance to infections The evidence is in agreement with the view of Gentles and Holmes[9] that some breakdown in personal immunity must precede the onset of clinical disease, and that this breakdown must coincide with inoculation by a viable fungus Such a coincidence is more likely to occur if the subject is in daily contact with infective particles either in his home or elsewhere

It appears possible that *T. rubrum* is, in fact, less infectious than *T mentagrophytes*, and that it owes its spread in recent years more to the persistence of clinical infections than to any unusual degree of infectivity This fungus is more likely to become a nuisance in a family, a residential home, in barracks, or pit-head baths used daily by the miners, than in such places as swimming baths and sports pavilions which are mostly frequented only once or twice a week by any one person (athletes, swimming instructors and similar persons must obviously be excepted) In a recent, unpublished survey carried out in day schools in Bristol, only 13 cases of tinea pedis due to *T rubrum* were found as against 154 due to *T mentagrophytes*, and there were never more than 2 cases of *T rubrum* infection in any one school

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cause of dermatophytosis in temperate climates Surveys of sections of the public who may not have requested medical advice show that *T. mentagrophytes* is still the predominant cause of ringworm, but as the symptoms it causes are less chronic and disfiguring, its victims seek medical aid less often.

There is little doubt that the two world wars and the consequent wholesale movements of troops and civilian populations have contributed to the spread of *T rubrum* It has probably been aided by the consequent increase in communal living, often in very crowded conditions, by lack of medical attention and, according to some workers[12], by certain predisposing diseases. An important factor, and one in which *T. rubrum* differs in degree from other dermatophytes, is its great resistance to treatment, particularly once the nails have become involved; an infected person is likely to be a source of infection for others for the rest of his life.

INVESTIGATION OF SOURCES

Many patients are unable to account for their infection in the ways referred to above, and neither have they been regular frequenters of swimming baths or sports grounds

It was partly in an attempt to find other sources of infection that from 1955 to 1956 an investigation was carried out of Bristol families, in each of which one person was known to be infected with *T rubrum* The families were visited in their homes in order to examine the feet and hands of each member, to record relevant personal and family histories, and to obtain scrapings from all suspicious lesions A spread of infection within the family was recorded only when mycologically similar cultures of *T rubrum* were obtained from two or more persons in it In 19 families concerned, a spread of infection was recorded in 9, and of the 48 contacts seen, 13 (27 per cent) were infected The rate of spread was very high among children brought up in infected families, for of the 8 subjects between the ages of 14 and 35 who had been exposed as children, 6 had contracted the disease The minimum duration of exposure before clinical infection occurred was 1 year and the maximum 15 years Two wives who had been in contact with their infected husbands for 30 years had not contracted the disease. Unhygienic standards seemed to have little to do with spread of infection, for over half the families were of middle class, and in the only really filthy household among the remainder, only one of 7 contacts was infected

Seven of the original patients could trace their infections to residence or active service abroad, and in 4 others the disease was

TINEA PEDIS IN MINERS

J. G HOLMES

IN 1911 the first pithead bath was constructed in Britain In the same year, a joint meeting of British and French dermatologists was held at the Royal Society of Medicine in London to discuss "Eczematoid Ringworm of the Extremities". The Chair was taken by Malcolm Morris and the opening speakers were Sabouraud and Whitfield Assuming that white soggy skin between the toes was evidence of tinea pedis, Malcolm Morris gave as his opinion that ringworm of the extremities was common; Whitfield, on the other hand, considered it to be rare

Prosser White, who lived and worked in Wigan near a pithead bath which has been in continuous use since 1913, made no mention of ringworm in miners in the 1939 edition of his well-known book The first description of this condition was made by Knowles[3] who, in 1943 while in general practice in a colliery village, wrote a thesis in which he expressed the view that tinea pedis was a new disease of the miners He attributed this to cross-infection in the bath-houses which had been erected for the first time in his district only a few years previously

In 1951, Riddell[4] referred to tinea pedis in miners and considered that the problem arose not so much by cross-infection but by a change in susceptibility of the skin to infection brought about by bathing

EPIDEMIOLOGICAL FIELD SURVEY

It was about this time that the Industrial Epidermophytosis Committee of the Medical Research Council was constituted It was decided that a field survey should be carried out to study the epidemiology of tinea pedis Gentles and Holmes[1, 2] were appointed as mycologist and clinician respectively and work began with a pilot survey at pits which had baths These pilot studies confirmed that tinea pedis was common and that it was necessary to have laboratory corroboration of clinical diagnoses

The survey proper lasted throughout 1953 and 1954 During that time 12 pits and 2 power stations of the British Electricity Authority were visited It was not possible to select these at random but the places chosen were widely separated geographically The

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these were recorded as similar on the two occasions in 63 subjects, as more severe on the first examination than the second in 16, and as more severe on the second examination in 20.

TABLE 6

DETAILED LABORATORY FINDINGS

Ringworm fungi	Microscopy +	346 (79 per cent)	403 cultured (92 per cent of total infected)
	Culture +		
	Culture only +	57 (13 per cent)	
	Microscopy only +	32 (7 per cent)	
Candida albicans		2	
Doubtful microscopy		1	
Total personnel infected		438 (21 per cent of those examined)	

At one pit which had the highest incidence rate for tinea pedis, a further examination was made about 1 year after the first. The results for 80 of the 99 men re-examined remained unchanged, 46 being infected on both occasions and 34 being apparently free of disease both times Ten were positive for fungous infection on the first occasion only and 9 on the second only. The total infected at one time or another was 65.

EFFECT OF ENVIRONMENT

The prevalence rates of infection proved by laboratory findings varied from 3 5 per cent at one pit to 50 per cent at another[1 (Table 1)]. The incidences at 2 pits in Scotland were similar as were those at 2 power stations in London On the other hand, results at some neighbouring places were dissimilar (for example, 50 per cent at one compared with 12 per cent at another) It was, therefore, necessary to look for reasons which would explain why this occurred, such as seasonal factors, conditions peculiar to mining, or the presence of pithead baths

Seasonal variation was ruled out as a factor from a study of results over the period of the survey When attention was paid to the effect of mining conditions, it appeared at first that those who worked underground were most likely to contract infection The incidence of tinea amongst surface workers who had never been underground was 12 per cent compared with 17 per cent in those who had, and with 24 per cent in underground miners This last figure is, however, no higher than that recorded at power stations, and all the apparent differences disappeared when the bathing histories of

personnel included in the trial at each pit or power station were selected at random from wage sheets using tables of sampling numbers; they were notified and given appointments in writing. The response rate was satisfactory. of the 2,160 selected, 97 per cent were seen, 2 per cent were not readily available and only 1 per cent refused to co-operate.

History-taking and physical examination were standardized as far as possible and the results entered on a record card. Approximately 5 minutes was the maximum time available for these examinations Skin scrapings were taken during this time from each foot irrespective of the clinical findings. The scrapings were wrapped in numbered slips of paper and posted off daily to the mycologist, who remained unaware of their origin until after he had made his report. The material was cultured in every case and whenever possible was also examined microscopically "Mosaic fungus" was not counted as evidence of infection.

CLINICAL AND LABORATORY FINDINGS

Only 10 per cent of the men examined had clinically normal feet and of these 2·5 per cent were found by laboratory tests to be infected. Infection of the feet as assessed from mycological examination was recorded in 21 per cent of subjects The detailed laboratory results are shown in Tables 6 and 7 and clinical findings have been described elsewhere[2] *Trichophyton rubrum* predominated greatly at one pit and at another no case of infection by this organism was detected

While conducting this survey an attempt was made to assess observer error When the laboratory findings were negative in a subject suspected clinically as being infected, a further examination was made of surplus skin material left over from the initial tests It was not possible to obtain fresh material from such cases owing to the extensive travel from place to place which this survey entailed From 98 such re-examinations, only 1 extra case of infection was recorded From a clinical standpoint, 99 men were examined on two occasions and the results compared These men were taken in three separate groups of 30, 34, and 35 respectively, and were examined while sitting behind a screen so that only their legs were visible. On the first occasion, the subjects were seen in any order, and on the second in order of random selection In the first series of examinations 18 were recorded as having normal feet compared with 20 at the second examination Ninety-three of the 99 men were classified in the same way on the two occasions, including 16 normals Degrees of abnormality proved more difficult to assess;

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personnel were taken into account. Similarly, though the peak incidence of infection in the groups studied occurred at the age of 45 years, the fall in incidence of tinea which was observed after this age coincided with a rise in the proportion of "non-bathers" in the older age groups Men who used the baths at places visited in the survey are referred to as "bathers" and the others as "non-bathers". The regularity of bathing had little effect on the figures for incidence of tinea provided a man bathed at least once weekly. Past bathing history had little influence on the infection rate amongst bathers but affected greatly this incidence among non-bathers[1 (Table 2)]. The rates at pits without baths depended very much on whether or not there were other baths in the vicinity.

TABLE 7

SPECIES ISOLATED FROM 403 SUBJECTS FROM WHOM RINGWORM FUNGI WERE GROWN

Trichophyton mentagrophytes	224
Trichophyton rubrum	148
Epidermophyton floccosum	11
T mentagrophytes and *T rubrum*	13
Ep floccosum and *T rubrum*	4
Ep floccosum and *T mentagrophytes*	3
Total	403

Length of employment could be used as a rough measure of length of exposure. The infection rate rose relatively steeply over the first few years of employment (a rise not apparent over weeks or months) before becoming stabilized. Similar conclusions were reached by Vanbreuseghem, Peeters and Tritsmans in their studies[5]

Gentles was successful in isolating *T rubrum* from the floor of one bath-house and *T mentagrophytes* from floors at various other places Measures designed to prevent spread of infection proved useless probably because they were undertaken too late

By these investigations it was shown that it is possible to obtain a reasonable estimate of the incidence of tinea pedis in a community even when, as in mining groups, only a limited time for interview and examination is available It was concluded that while personal immunity is an important factor in this disease, exposure is of even greater moment Though common, tinea pedis is, fortunately, seldom disabling

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extract was washed repeatedly with water until no traces of acetic acid remained It was then concentrated by evaporation under reduced pressure to about one-sixth of its original volume, further concentration resulting in precipitation of the pigment.

A small quantity of this extract, about 50 microlitres, was streaked across a 2-inch strip of Whatman No. 3MM paper for chromatography and the paper was then placed in a mixture of 2 parts chloroform to 1 part petroleum ether (b p. 60–80°). The composition of this mixture was critical for satisfactory separation of the pigments and by using it 3 pigments were clearly distinguished The fastest-moving pigment (A) is bright yellow, the second (B) is an orange-red pigment and the third is a deep bluish-red pigment (C) Another very faint yellow band can be seen travelling more slowly still, but the amounts of this are very small.

The three main pigments were eluted from the paper with chloroform and their colour noted when alkali was added Pigment A turned mauve, pigment B turned violet, and pigment C turned blue When reduced in alkaline solution the colours of the pigments change, decrease greatly in intensity, and become fluorescent, A, B and C having a green, a yellow and a yellowish-green fluorescence respectively These reduced forms are unstable and readily revert to the oxidized forms in air

The separation of these pigments on a larger scale was effected in the following way Petroleum ether was added to the chloroform extract, thereby precipitating pigments B and C but leaving pigment A in solution This solution was evaporated to small bulk and cooled but so far crystals of pigment have not been obtained, probably due to the presence of contaminating fatty material Pigment B was obtained by dissolving the precipitate in chloroform and carrying out repeated fractional precipitation with methyl alcohol until only one pigment could be detected on the chromatogram, this pigment has been obtained in crystalline form The final supernatant was evaporated to dryness under reduced pressure and the residue was dissolved in the smallest possible volume of hot 90 per cent acetic acid When this was allowed to cool, pigment C crystallized out as sheaths of fine purple needles

The absorption spectra of these 3 pigments have been studied and have been found to be very closely related. Their spectra, the colour reactions, the solubilities and other properties strongly suggest that these pigments are substituted anthraquinones. Most of the fungal pigments which have been isolated are, in fact, anthraquinones and most of them have contained at least 2 substitutes Since the particular spectra observed by Mier have not been recorded

PIGMENTS OF *TRICHOPHYTON RUBRUM*

A TICKNER

ALTHOUGH fungal pigments in general have been quite extensively studied, chiefly by Raistrick and his colleagues, not much is known about the pigments produced by pathogenic fungi The earliest work was by Tate in 1929[2] who described what he thought was a single substance produced by 5 species of dermatophytes apparently including *Trichophyton rubrum* although this was not mentioned by name He described the properties of the crude material as a red to reddish-brown substance in its natural state, soluble in dilute acid and in acid-alcohol but not in dilute alkali In acid solution it was yellow, becoming red on addition of alkali It could be extracted from acid solution by chloroform. Alkaline solutions could be reduced to a clear yellow solution with sodium dithionite; the reduced solution was re-oxidized spontaneously and rather quickly by atmospheric oxygen Guided by his biochemical colleague Hill, Tate made the percipient observation that the substance appeared to be an anthracene pigment

Thompson[3] stated that the pigment (*sic*) of *T purpureum* (*T. rubrum*) and of *T gypseum* (*T mentagrophytes*) was readily soluble *in acetone Oxygen caused it to change to a bright cherry colour*, and addition of water caused partial precipitation of the pigment. The soluble portion was cherry coloured, turning lavender if the *p*H was above 9 On standing in alkaline solution the pigment turned yellow, but the lavender colour could be restored by the addition of oxygen but not nitrogen, hydrogen sulphide or carbon dioxide

ISOLATION OF PIGMENTS

An attempt was made to isolate and identify these pigments, in the chemical pathology laboratory of the Institute of Dermatology, by Mier[1] Cultures of *T rubrum* grown on 50-millilitre plates of Sabouraud's medium were sterilized by heat and the pooled contents of 12 plates were then scraped into a mortar where they were thoroughly ground in about 250 millilitres of glacial acetic acid By protracted grinding it was possible to extract all of the pigment originally present in the plates This extract was diluted to 2 litres with water The pigment could be extracted from the glacial acetic extract by chloroform and was a reddish colour The chloroform

extract was washed repeatedly with water until no traces of acetic acid remained It was then concentrated by evaporation under reduced pressure to about one-sixth of its original volume, further concentration resulting in precipitation of the pigment.

A small quantity of this extract, about 50 microlitres, was streaked across a 2-inch strip of Whatman No 3MM paper for chromatography and the paper was then placed in a mixture of 2 parts chloroform to 1 part petroleum ether (b p 60–80°) The composition of this mixture was critical for satisfactory separation of the pigments and by using it 3 pigments were clearly distinguished The fastest-moving pigment (A) is bright yellow, the second (B) is an orange-red pigment and the third is a deep bluish-red pigment (C) Another very faint yellow band can be seen travelling more slowly still, but the amounts of this are very small

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The absorption spectra of these 3 pigments have been studied and have been found to be very closely related Their spectra, the colour reactions, the solubilities and other properties strongly suggest that these pigments are substituted anthraquinones Most of the fungal pigments which have been isolated are, in fact, anthraquinones and most of them have contained at least 2 substitutes Since the particular spectra observed by Mier have not been recorded

elsewhere, it is quite possible that these particular substituted anthraquinones are peculiar to the *Trichophyton* genus and perhaps to the *T rubrum* species

Mier has also carried out preliminary studies on *T. violaceum* and has shown, again by paper chromatography and by plotting the absorption spectra, that there are at least 3 pigments and perhaps as many as 7 or 8. These pigments are very similar to those of *T. rubrum* and also appear to be substituted anthraquinones He has investigated the pigment of one other fungus, *Microsporum canis.* This pigment was very different from the *Trichophyton* pigments and was much more difficult to isolate, present indications are that it is an unsaturated dicarboxylic acid

As to the reason why the trichophyta are so lavish in the production of pigments, two possibilities suggest themselves. First, since the anthraquinones are manifestly able to act as electron acceptors they could take some part in oxidation-reduction mechanisms in the respiratory chain It may be that under certain circumstances this particular group of organisms may make too much pigment for their own needs, analogous to the excessive production of riboflavine by *Eremothecium ashyi* Anthraquinone co-enzymes appear, on the other hand, to be unknown Alternatively, it is possible that the trichophyta may secrete substances harmful to other fungi and thus be more successful in their struggle for existence. This effect has not been demonstrated with these pigments, but a preliminary screening for bactericidal action was negative It is also possible that fungi living under unusually rich growth conditions may produce pigments in the same way as the overfed human produces fat

The work described was carried out with very small quantities of material, but conventional chemical analysis is awaiting the availability of larger amounts

Addendum —Since this work was completed we have communicated with Dr J C Wirth of Brooklyn[4] who has been carrying out similar investigations, in particular he has been successful in producing a pure specimen of pigment A

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IMMUNITY IN YEAST INFECTION

H. I. WINNER

THE STUDY of infections due to fungi has mainly concerned itself with the recognition and systematic study of etiological agents and of clinical syndromes. Immunological aspects of these diseases have received little attention, and there has been a tendency to borrow ready-made concepts from bacterial infections In the literature there are accounts of human moniliasis treated with antimonilial rabbit serum on assumptions deduced from certain bacterial and virus infections On similar assumptions, attempts have been made to actively immunize against blastomycosis by injecting killed vaccines of the causative fungus Such classical immunological concepts cannot, however, be applied to all bacterial infections; for example, one does not try to immunize against *Treponema pallidum* infection in this way

An important difference between fungal and bacterial infections is the relative size of the infecting organisms. It can be assumed that the infecting agent in bacteriological and virus diseases is smaller than the smallest host cells, small enough to pass between cells, across cellular membranes such as the glomerular basement membrane without causing appreciable mechanical disturbance, and small enough to pass across capillary walls In the case of the fungi such assumptions cannot be made Pathogenic yeasts, like *Candida albicans* and *Cryptococcus neoformans*, have approximately the diameter of an erythrocyte and a volume which is usually greater Their very physical existence in the host, were they merely inert particles, would raise mechanical problems by virtue of size alone, problems which do not arise in the case of bacteria and viruses except in the most unusual circumstances The proliferating yeasts of *C albicans* form large masses inside the host tissues and these organisms may also produce filamentous structures (Fig 61)

There is a further difference between fungi and bacteria in the nature of their cell walls The tough polysaccharide wall of yeasts might well be expected to render such cells resistant to damage by the protective mechanisms of the host. The yeasts are likely to be relatively immune to the digestive action of the host enzymes, to surface-acting antibodies, to such agents as lysozyme, and also probably to phagocytosis

Again, fungi are different in being saprophytic organisms with a complex metabolism involving enzyme systems more complex than those of the smaller micro-organisms The existence of such systems operating inside a metazoan host would itself create special biochemical problems. The fungal cell, by virtue of its size as much as anything else, probably possesses a very large number of antigens.

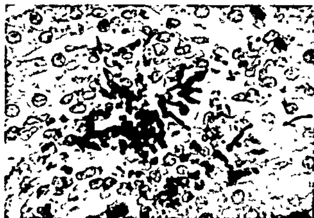


FIG 61 —Section showing yeasts proliferating in tubule of rabbit kidney 24 hours after infection with *C albicans* Note absence of inflammatory response (×392 Periodic acid Schiff and haematoxylin)

Since a cell of *Salmonella*, with a volume of the order of $1\mu^3$, contains a battery of several flagellar antigens and another of somatic antigens, it is likely that a yeast cell, with a volume some hundreds of times as great, would be much more complex antigenically Skin hypersensitivity reactions to whole fungus, or to extracts of fungus, can often be demonstrated in mycotic infections, though their significance is not understood and their occurrence is inconstant

formans, provide an instructive contrast in this aspect of immunology.

INNATE IMMUNITY

C albicans is best regarded as a normal commensal organism of humans and of many other animals This is amply shown by the

frequency with which it is isolated from the skin and mucous membranes of human subjects[4], and by the frequency with which agglutinins to it can be demonstrated in the absence of clinical infection. Humans are normally resistant to the pathological effects of *C. albicans*, but occasionally resistance is lost and clinical disease results Resistance is also shown by laboratory animals infected experimentally The intradermal injection of large doses of *C. albicans* cells into rabbits gives rise to a self-limiting disease The organism spreads locally, exciting a cellular response, and an abscess is produced, the multiplying organism is extruded and within a few weeks the lesion heals The intraperitoneal injection of massive doses [illegible] visceral organs

Intravenous injection of *C. albicans* cells, on the other hand, may cause a fatal infection in rabbits, guinea-pigs, and mice The establishment of disease in the rabbit has long been regarded as pathognomonic of the presence of *C albicans* in injected material The disease is, however, only fatal if the infecting dose of pathogenic organisms exceeds a definite threshold[11], the rabbit having a degree of natural immunity which can be overcome by force of numbers of pathogenic fungus cells Intravenous injection of *C albicans* in doses of 2 million organisms or less fails to produce death, whereas injections of 5 million organisms or more almost invariably do so (Table 8)

TABLE 8

Number of animals	*Organisms received*	*Result*
24	2 million or less each	All survived
43	5 million or more each	40 died within 7 days, 1 died after 18 days, 1 showed progressive disease after 30 days, 1 survived

In the case of *Cr neoformans*, it is uncertain whether a permanently subclinical infection with this organism occurs in humans Almost all of the clinical cases, which are coming to light in increasing numbers, ultimately prove fatal, and several cases have been reported of disease progressing to death from a comparatively trivial initial lesion When any relatively rare and often fatal infection is

gaining recognition, most of the early cases reported are fatal ones In consequence, the existence of subclinical disease tends to be overlooked. Weidman in 1935 coughed up an organism identified as *Cr. neoformans* and 15 years later, alive and well, he reported the fact[9]; it is possible that this represented a subclinical infection or, alternatively, the organism may have been a non-pathogenic variant. Many of the clinical cases of torulosis reported have been complications of some debilitating condition such as Hodgkin's disease or sarcoidosis, or of cortisone therapy with its well-known depression of immunity mechanisms

There are well-marked differences in susceptibility of other animals to *Cr neoformans* infection. Mice are extremely susceptible, particularly the white Swiss strain, and most strains of *Cr neoformans* derived from human cases will kill these animals within 21 days of intraperitoneal or intracerebral injection of the organism. Rats, guinea-pigs and rabbits show an increasing gradient of resistance It has been suggested that the relatively high resistance of the rabbit to this infection is due to the high body temperature (39·5° C.) of this animal, particularly as the organism itself shows a marked susceptibility to heat

ACQUIRED ACTIVE IMMUNITY

Many humans and rabbits possess agglutinins to *C. albicans*[10] (Tables 9 and 10) There have been several other surveys in the Western Hemisphere of human agglutinins[1, 3, 6, 8]. In the majority of cases such agglutinins are probably acquired, though the possibility of their being natural agglutinins cannot be ruled out Females show agglutinins more often than males, suggesting that these

TABLE 9

CANDIDA ALBICANS AGGLUTININS IN SERA OF HOSPITAL PATIENTS

	Number tested	*Number positive*	*Per cent positive*
Antenatal cases	1391	445	32 0
Other females	264	94	35 6
Males	362	99	27 3
TOTALS	2017	638	31 6

TABLE 10

NATURAL AGGLUTININS TO *CANDIDA ALBICANS* IN RABBITS

(Number tested: 55)

No agglutinins detected in	36 (65%)
Some agglutinins detected in	19 (35%)
Titre less than 16 in	44 (80%)
Titre 16 or higher in	11 (20%)

antibodies are, in fact, acquired, since moniliasis occurs more frequently in females. As Norris and Rawson[6] have shown, there is much cross-immunity associated with various species of *Candida*, so these agglutinins are by no means necessarily specific.

It is important to establish whether the acquisition of agglutinins confers any resistance to the pathogenic effects of *C. albicans* This has been studied in rabbits Agglutinin formation is easily induced in these animals, either by single or multiple injections of sublethal doses of living organisms or by repeated injections of killed organisms. Whichever method is used, agglutinins develop[11] with titres ranging from approximately 1/32 to 1/320, but when the animals are challenged with a dose of *C albicans* normally lethal to the rabbit, the acquisition of agglutinins does not protect them[11] This observation apparently departs from the classical conception of immunity based on bacterial and virus studies, but the type of disease found in rabbits dead of systemic moniliasis has little in common with that seen in animals dead of a systemic bacterial disease, such as staphylococcal septicaemia, typhoid or tuberculosis During the course of experimental infections the animals become less and less active, and may develop pareses and convulsions Before death, or at death, they show a raised blood urea The striking feature at post-mortem examination is the enlargement of the kidneys, macroscopically the lymph nodes and spleen appear normal and signs of septicaemia are absent However, sections of the kidneys, and of other organs too, show that the *C albicans* cells are multiplying freely in the tissues with or without the occurrence of a marked inflammatory reaction (Figs. 61, 62 and 63) A feature of the kidney lesions is the fibrinoid type of necrosis seen in the glomeruli[2] (Fig 64)

The size of the infecting *C albicans* cells is an obvious mechanical factor to be considered Living organisms, about 7μ in diameter, proliferate in the kidneys to produce large masses of cells, and the animals die in uraemia However, death is not due simply to glomerular blockage If it were, one would expect injections of killed *C albicans* to produce similar effects, whereas, in fact, they do



FIG 62 —Section showing the distribution of *C albicans* abscesses in the rabbit kidney. (×2 3 Periodic acid Schiff and haematoxylin)



FIG 63 —Section showing marked inflammatory reaction in kidney of rabbit infected with *C albicans* (×85 Haematoxylin and eosin)

not Massive doses of formalin-killed organisms injected intra-

injected, either in the lungs, spleen, kidneys, or lymph nodes

Furthermore, in contrast to what happens with *C. albicans*, the injection of comparable doses of living cells of other *Candida* species (for example *C. tropicalis*) is not lethal, as might be the case if purely mechanical factors were operating.

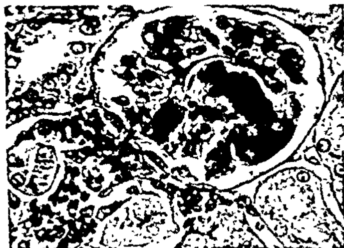


Fig 64.—Section showing amorphous deposit in glomerulus of kidney of rabbit infected with *C albicans* (× 550 Periodic acid Schiff and haematoxylin)

Henrici[5] was the first to postulate that *C albicans* elaborated a toxin, and though others have supported this hypothesis no toxic substance has yet been isolated The cellular necrosis in the kidneys of animals dead from experimental moniliasis suggests either the action of a toxin or the occurrence of a local hypersensitivity reaction

It is possible to produce erythema in the skin of rabbits by the injection of simple saline extracts of broth cultures of *C albicans* This also suggests that a toxin may be present and may indicate why the production of agglutinins does not appear to coincide with resistance

Few attempts have been made to produce acquired immunity against experimental systemic infection with *Cr neoformans* Agglutinins, precipitins, and complement fixing antibodies

against this organism have been detected, not without some difficulty, both in patients with natural infections and in animals experimentally infected. Recently, complement fixing antibodies have been detected in actively immunized rabbits, using as antigen a heated ether-alcohol extract of alcohol-killed *Cr neoformans*[7]. It remains to be seen whether the possession of these antibodies confers any resistance against *Cr. neoformans* infection An extensive survey of natural antibodies against this yeast in humans and animals not known to be infected by it is required

Cr neoformans usually has an abundant capsule when freshly isolated from lesions, but this tends to be lost in subcultures The possession of a capsule may contribute to an important degree to the virulence of this organism If the yeast is unencapsulated when free living and when acting as a commensal organism, but encapsulated when producing disease, interesting immunological problems at once present themselves

Finally, let us consider the conditions in which human disease due to *C. albicans* and *Cr. neoformans* is met with Infection with *C albicans* is virtually universal Disease due to this fungus occurs in certain well-defined conditions which were characterized by Trousseau in his studies of thrush in Paris from 1843 onwards as "a local expression of a very bad state of the whole organism" Normally, the multiplication and spread of this yeast-like organism is held in check by the natural defence mechanisms of the body, but the following host factors have been observed to enhance susceptibility to disease

(1) Infancy
(2) Debility
(3) Interference with immunity mechanisms
  (*a*) Systemic disease (for example Hodgkin's disease, leukaemia)
  (*b*) Steroid therapy
  (*c*) Depression of bone marrow activity
  (*d*) Irradiation of whole body
  (*e*) Drug addiction
(4) Hormonal changes (diabetes, pregnancy, and so on)
(5) Interference with the normal bacterial flora of the body (for example, effects of antibiotic therapy)
(6) Mechanical or chemical damage to mucous membranes (due to smoking and so on)

Practically nothing is known of natural fluctuations in virulence of *C. albicans* from strain to strain, but one would expect these to exist.

With regard to *Cr. neoformans*, many cases of human infection have been associated with debilitating conditions, particularly those affecting immunity mechanisms such as Hodgkin's disease, or with steroid therapy. On the other hand, when freshly grown from lesions, the yeast possesses a capsule which has antigenic properties. It appears likely that in *Cryptococcus*, in contrast to *Candida* infections, variations in virulence of the fungus play a large part in the determination of disease.

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FUNGOUS INFECTIONS FOLLOWING ANTIBIOTIC THERAPY

T. ANDERSON*

STUDIES on *Candida albicans* infection in patients suffering from respiratory infections were carried out before and after antibiotic therapy.

The occurrence of *C. albicans* was estimated by rectal, throat and sputum cultures at the time of admission to hospital (Table 11) When the incidence of *C. albicans* in the sputum of various groups of patients was compared (Table 12), it was found that the proportion of patients in the various clinical categories harbouring this organism, even in large numbers, was similar. There was a trend in both sputum and saliva towards a poorer yield of yeasts as the *p*H rose, but this difference was not within the level of statistical significance.

Administration of antibacterial drugs permitted an overgrowth of fungi[3] which were insusceptible to the action of these agents (Tables 13 and 14). Sulphonamides seemed to be less liable to cause this After treatment with a tetracycline the proportion of patients giving a heavy growth of *C albicans* from sputum was almost doubled, and in the faeces the increase was very much greater[4]

The results left no doubt that after treatment with antibiotics it was comparatively easy to isolate *C albicans* from sputum or faeces This ease of isolation did not mean, however, that the fungi were playing a pathogenic role in all cases[1, 2]. In order to study this aspect of the problem, 155 patients were observed during treatment for side-effects (Table 15) A number of patients experienced gastric

TABLE 11

ISOLATION OF *CANDIDA ALBICANS* FROM RECTUM, THROAT AND SPUTUM OF PATIENTS NEWLY ADMITTED TO HOSPITAL

	Rectal swab	*Throat swab*	*Sputum*
Total examined	362	291	367
Total showing heavy growth of *C albicans*	12	36	119 (32 per cent)

* With the co-operation of Jessie L Sharp and A J Childs

TABLE 12

PRESENCE OF *CANDIDA ALBICANS* IN THE SPUTUM IN VARIOUS GROUPS OF PATIENTS

	Total patients	*Results of culture for* C albicans	
		+	++
			percentage
All patients	367	60	119 (32)
Patients under 40 years of age	110	22	30 (27)
Patients over 40 years of age	257	28	89 (34)
Chronic bronchitis	138	25	47 (34)
Tuberculosis	57	7	24 (42)
No history of chest disease	172	30	47 (28)
Definite pneumonia	208	33	62 (30)
Cor pulmonale	33	6	9 (27)

TABLE 13

ISOLATION OF *CANDIDA ALBICANS* FROM SPUTUM BEFORE AND AFTER CHEMOTHERAPY

	Tetracyclines		*Sulphonamides*		*Tetracycline + Nystatin*	
When patient examined	*Number of patients examined*	*Heavy growth of* C albicans	*Number of patients examined*	*Heavy growth of* C albicans	*Number of patients examined*	*Heavy growth of* C albicans
On admission	53	13 24 5 per cent	33	12 36 4 per cent	25	4 16 per cent
3rd–5th day	53	25 47 1 per cent	33	10 30 per cent	25	6 24 per cent
7th–9th day	53	23 43 5 per cent	33	16 48 1 per cent	25	7 28 per cent

disturbances but this was most often co-related with the dose, form and frequency of administration of the antibiotic itself The diarrhoea which occurred fairly frequently also seemed unlikely to be connected with the presence of fungus for the following reasons

(*a*) It arose less frequently when the patient was on a diminished dietary intake For example, it was less frequently observed in

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(*b*) the antibiotics were responsible in different degrees. Oxytetracycline, for instance, appeared to be the most common cause, (*c*) a reduction of the amount or frequency of drug administration diminished the incidence of diarrhoea

Glossitis was often associated with an absence of *C. albicans* and the only side-effect which was in any way related to fungous infection in this series was black tongue Only one example of urinary infection by *C. albicans* has been seen in an elderly female patient Among approximately 1,000 patients receiving broad spectrum antibiotics studied mycologically, no example of serious effects attributable to *C. albicans* has been detected.

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TABLE 14
ISOLATION OF *CANDIDA ALBICANS* FROM RECTAL SWABS BEFORE AND AFTER CHEMOTHERAPY

	Tetracyclines		*Sulphonamides*		*Tetracycline + Nystatin*	
When patient examined	*Number of patients examined*	*Heavy growth of* C. albicans	*Number of patients examined*	*Heavy growth of* C. albicans	*Number of patients examined*	*Heavy growth of* C. albicans
On admission	110	2 1 8 per cent	69	2 3 1 per cent	25	2 8 per cent
3rd day	110	18 16 4 per cent	69	2 3 1 per cent	25	0
5th day	110	60 54 8 per cent	69	4 6 2 per cent	25	1 4 per cent
7th day	110	62 56 3 per cent	69	10 15 5 per cent	25	0
9th day	110	62 56 3 per cent	69	14 21 9 per cent	25	0

TABLE 15
SIDE-EFFECTS FROM TETRACYCLINES IN RELATION TO THE ISOLATION OF *C. ALBICANS*

Side-effect	*Number of persons with side-effects*	*Results of culture for* C albicans			
		Negative	+	+ +	*Origin*
Gastric disturbance nausea, vomiting or epigastric pain	19	3	3	13	Faeces
Diarrhoea	44	3	8	33	Faeces
Atrophic glossitis	16	9	3	4	Throat
Black tongue	7	0	2	5	Tongue
Ano-rectal syndrome	4	1	2	1	Anus

Total persons included in this study 155

pneumonia patients who at the early stage of treatment were on a fluid diet than in healthy patients on a normal diet, yet in both groups the frequency of isolation of *C albicans* was similar,

innumerable hospitals of otherwise good standing, rubber gloves are regarded as a protection only for the gynaecologist and not for the patient, so that the same pair are worn throughout a whole clinic without changing and boiling between cases.

AETIOLOGY

Pregnancy as an aetiological factor has already been mentioned This is thought to be due, in part at least, to the very highly acid state of the vagina in pregnancy. Glycosuria also provides a very favourable environment for *C. albicans*. It is difficult, however, fairly to compare the incidence in the pregnant and the non-pregnant state since obstetrical and gynaecological patients do not as a rule attend the same clinic. The carrier state has already been mentioned and it is suggested that some fresh local event or complication is necessary to induce pathogenicity. Infection may be subpreputial in the male and introduced thus, or the anus may provide another source of infection, but it is doubtful if the patient can become clinically infected simply by direct inoculation without an accompanying alteration in local circumstances such as the effects of trauma, the presence of glycosuria or the local effects of pregnancy.

A new source of mischief, namely the wide spectrum antibiotics, now requires consideration Stewart[8], for example, found that oral tetracyclines are particularly likely to encourage a *C albicans* infection in throat and sputum Fortunately this problem does not occur to any extent in the field of gynaecology, partly because, with the exception of pelvic tuberculosis, surgeons on the whole prefer to treat chronic pelvic infections by surgery rather than by a prolonged course of antibiotics.

CLINICAL PICTURE

Whether the patient is pregnant or diabetic, or neither, pruritus vulvae with superadded soreness is common to all clinically infected cases Discharge is present in the vast majority but is less universal than pruritus, which is the dominant symptom. These symptoms can reduce the sufferer to a state of sleepless misery in which she is driven to adopt almost ritual methods of "expurgation", going from one antiseptic to the next The clinical picture is often confused by local eczema which is the result of injudicious treatment. The infection can spread to the male partner, though usually in a very mild form, in the shape of a transient scrotal dermatitis In all these cases specimens of vaginal discharge are taken for smear and culture

VULVO-VAGINAL MONILIASIS

IAN DONALD

As a clinical problem vulvo-vaginal moniliasis has been with us in gynaecology for a long time, although in recent years it has come to [illegible] because [illegible] cannot [illegible] which vaginal discharges are nowadays investigated and the greater use now made of laboratory methods.

As a complication of pregnancy or of diabetes mellitus, infection of the vagina with *Candida albicans* has long been recognized. For instance, Liston and Cruickshank[6] recorded that 49 out of 200 cases of leucorrhoea in pregnancy were due to this fungus, and figures today would probably be higher. It is less generally recognized that monilial infections still account for a significant number of cases of vaginal discharge in the absence both of pregnancy and glycosuria. In 1952 a review of 585 cases of vaginal discharge at the Chelsea Hospital for Women showed the incidence of *C. albicans* to be 16·2 per cent, a surprisingly high figure[4] Diagnosis in all these cases was established microscopically and only 6 of the 96 cases in this series were in fact pregnant Out of 478 cases enrolled in the Leucorrhoea Clinic at the Western Infirmary, Glasgow, 36 cases have been proved due to *C albicans* by smear and culture, an incidence of 7 5 per cent Of these approximately half have glycosuria and none is pregnant

Proper laboratory investigation undoubtedly yields high figures of incidence since a clinically latent infection without symptoms is quite common; for example, Dawkins, Edwards and Riddell[3] investigating 500 cases attending a Family Planning Centre found yeasts in no less than 57, of which 38 were due to *C albicans* and the remaining 19 to 5 other species Only 6 of the patients had clinical signs of infection and all of these were *Candida* cases

It is certainly true that sufferers from this infection frequently enjoy symptomatic remissions while still showing positive cultures This raises the possibility of a carrier state and emphasizes the need for a scrupulous technique in vaginal examination to avoid the possibility of transference of infection, which is even more readily perpetrated in the case of *Trichomonas vaginalis* Unfortunately, in

examination and a note is also made of the pus/squame ratio, which often gives a better quantitative index of the clinical state than the bacteriological findings Cultures are made on Nickerson's medium (Ortho) and incubated at room temperature for 5 days. *Candida* colonies appear brown or black in colour and this diagnostic aid should be of great use to practitioners who are removed from full bacteriological services. Considering the ease with which these cases can now be treated a readily made diagnosis is very important. The discharge itself is not by any means always copious and can often be recognized by one or two forms: the curdy type, which may be likened to damp bread, and the more glairy type, which is usually more profuse The vagina may be considerably reddened and the vulva inflamed, excoriated, oedematous and eczematized. The skin changes often spread backwards around the anus Where profound and chronic skin changes are present leukoplakia enters into the differential diagnosis Any doubt on this matter justifies biopsy, bearing in mind the very appreciable risk of malignant change

TREATMENT

Many treatments have in the past aggravated the condition and produced sensitization phenomena, so that the last state of the patient was made worse than the first The only reliable treatment was with aqueous gentian violet (½–1 per cent) and even this was frequently prescribed in too great a strength, so that a type of burn, sometimes severe, resulted Painting out the vagina with this dye every day or two was more or less effective but relapses were common. The dye cannot be kept off underclothing and bed linen in spite of extravagant precautions and at every turn is likely to be a most demoralizing reminder to the patient of her complaint The pregnant cases fared best of all since, though their misery was often intractable, they nearly always rapidly got better after delivery

It is essential to test for glycosuria and to treat all cases of diabetes energetically Relapses of moniliasis in diabetics are nearly always associated with recurrences of glycosuria due to inadequate control

The general principles of treatment have lost none of their importance The really acute case requires admission to hospital, where not the least benefit is that the patient is thereby denied access to soap and water and so-called harmless antiseptics, all of which aggravate the local skin condition Local cleansing can more safely and satisfactorily be carried out with olive oil

The advent of Nystatin (derived from *Streptomyces noursei*) has

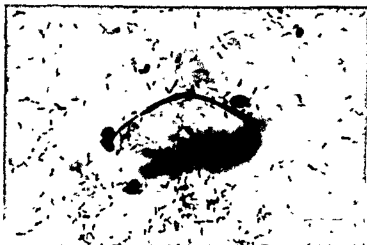


FIG 65 —*C albicans* in vaginal smear showing yeast cells and mycelial forms (× 1000 Stained Gram)

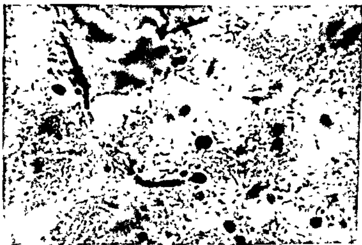


FIG 66 —Degenerate filamentous structures of *C albicans* in vaginal smear (× 1000 Stained Gram)

The cases which failed to improve fared no better with oral tablets of Nystatin (500,000 units) given for 5 days in a dosage of 2 tablets twice a day; a second course of local Nystatin was found highly effective, however, provided that any recurrent glycosuria in diabetic cases was dealt with at the same time.

The place of surgery in the treatment of really resistant cases must still be mentioned. There is no doubt that continuing associated pelvic pathology, for example, an infected cervix with cervical erosion, can encourage persistence or recurrence of monilial infection. In such cases surgical measures are indicated Diathermy conisation of the endocervix is a most valuable therapeutic measure in eliminating this source of reinfection.

ACKNOWLEDGEMENTS

The figures quoted above are given by courtesy of Dr Wallace Barr, in charge of the Leucorrhoea Clinic of the Western Infirmary, Glasgow, and the illustrations are provided by Dr I W. Lominski

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come as a very great therapeutic advance in this disease. Fortunately, this antibiotic does not appear to have any influence upon the natural flora of the vagina. Reports in this country by Stewart[8] and Childs in Glasgow[2] on the use of Nystatin in infections of the upper respiratory tract were soon followed by impressive accounts of its use locally in the vagina[7, 5, 1]. Symptoms were often relieved after the first day of treatment and results were, in general, better than those obtained in a control series treated with 1 per cent aqueous gentian violet There were no side effects due to Nystatin A week's course of treatment with pessaries containing 100,000 units of Nystatin is recommended; in 92 per cent of cases complete relief is initially obtained, often within a few hours. After a week's rest from treatment, further cultures are taken in order to detect the relapsing cases Tables 16, 17, and 18 show the results of treatment with Nystatin in the three groups: (1) pregnant; (2) non-pregnant and without glycosuria; (3) diabetic.

TABLE 16

PREGNANT SUBJECTS WITH MONILIASIS TREATED WITH NYSTATIN

No of cases	76
No with glycosuria (2 diabetics)	14
Effect of treatment	
Complete cure (bacteriological and symptomatic)	64 (84%)
Failure bacteriologically (but 5 symptomatic cures)	12
Relapse	10

TABLE 17

NON-PREGNANT SUBJECTS WITHOUT GLYCOSURIA SUFFERING FROM MONILIASIS AND TREATED WITH NYSTATIN

No of cases	18
Effect of treatment	
Complete cure	13
Symptomatic cure	1
Relapse	4

TABLE 18

DIABETIC SUBJECTS WITH MONILIASIS TREATED WITH NYSTATIN

No of cases	16
Effect of treatment	
Cured	12
Failure (2 with leukoplakia)	4

The cases which failed to improve fared no better with oral tablets of Nystatin (500,000 units) given for 5 days in a dosage of 2 tablets twice a day; a second course of local Nystatin was found highly effective, however, provided that any recurrent glycosuria in diabetic cases was dealt with at the same time.

The place of surgery in the treatment of really resistant cases must still be mentioned. There is no doubt that continuing associated pelvic pathology, for example, an infected cervix with cervical erosion, can encourage persistence or recurrence of monilial infection. In such cases surgical measures are indicated. Diathermy conisation of the endocervix is a most valuable therapeutic measure in eliminating this source of reinfection.

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[illegible] Nystatin in [illegible]

CANDIDA ALBICANS IN VAGINAL SECRETIONS IN PREGNANCY

SYLVIA M. DAWKINS, JOAN M. B. EDWARDS AND YVONNE M. CLAYTON

VARIOUS observers have reported that yeasts, particularly *Candida albicans*, occur more frequently in the vagina during pregnancy than at other times[3, 9]. Liston and Cruickshank[6] concluded that the incidence of moniliasis was greatest in the later stages of pregnancy, and Bland, Rakoff and Pincus[2] noted that infection by *C. albicans* was more readily established in the vagina during pregnancy. The raised glycogen content of the vaginal epithelium rather than greater acidity of secretions has been held mainly responsible for this increase in susceptibility[4] After parturition, yeasts disappear for a time from the vagina[7, 8] and it has been estimated that less than 10 per cent of infected pregnant women suffer from clinical recurrence of moniliasis during the post-natal period[1].

ANTE-NATAL AND POST-NATAL INVESTIGATION

Since no detailed investigation of yeast infections in pregnancy had been made in Britain, it was decided to carry out such a study in the Obstetric Unit of University College Hospital, London This was similar in plan and conduct to the survey of non-pregnant patients attending four family-planning clinics reported by Dawkins, Edwards and Riddell in 1953[5]

The first 3 or 4 patients attending the ante-natal clinic each week for examination at the sixteenth week of pregnancy were included in the investigation until a total of 250 was registered Subsequently, as many of these patients as possible were followed up at approximately the twenty-eighth and thirty-sixth weeks of pregnancy and the eighth or tenth day post-natally Swabs were also taken from babies' mouths One medically qualified person was responsible for the great majority of the clinical observations and for taking specimens; deputies played only a very small role Post-natal and babies' mouth swabs were taken by ward sisters. Clinical data were entered on a record card allotted to each patient, and culture results were recorded quite separately in order to avoid observer

bias Age, parity and past clinical history were recorded. The result of routine examination for sugar in the urine was noted; diabetics were excluded from the investigation

As in the previous survey, it was necessary to pose a direct question about pruritus since patients show a reluctance to volunteer information upon this symptom unless it is distressing, possibly because many associate itching with lack of cleanliness. This reluctance is perhaps less explicable during pregnancy when greater attention tends to be paid to untoward symptoms. Care was taken that patients did not confuse soreness, burning sensation or frequency of micturition with pruritus vulvae; other symptoms, including pruritus ani, were also noted

Methods of examination.—At each stage examination consisted of inspection of the vulva and swabbing of the lower vagina and of the vaginal pool for culture purposes Sabouraud's dextrose agar and penicillin-streptomycin blood agar plates were inoculated on the same day as swabs were taken. Each plate was incubated for 48 hours at 37° C and the species and amount of any yeasts grown were recorded as in the previous survey. *Only at 16 weeks was a speculum passed so that a note could be made of the condition of the vagina and cervix.* Unfortunately, a number of patients were lost to the survey from various causes, such as premature birth, admission to hospital for various reasons, miscarriage, and change of day of attendance and address Of the 250 patients registered, 217 were examined at all three stages of pregnancy.

TABLE 19

OCCURRENCE OF YEAST-LIKE FUNGI IN VULVAL AND VAGINAL SWABS AT SOME STAGE OF PREGNANCY

Yeast species isolated	*Number of patients*
Candida albicans	50 (20 per cent)
C guillermondi	3
C tropicalis	1
C pseudotropicalis	1
Torulopsis glabrata	11 (4 4 per cent)
Rhodotorula sp	3
Pullularia sp	1
Sporobolomyces sp	1
Unclassified	4
Total for all yeasts	75 (30 per cent)

TABLE 20

CULTURE RESULTS AT 16, 28 AND 36 WEEKS OF PREGNANCY AND POST-NATAL

Stage of pregnancy	*Number of patients*	*Yeasts cultured*		
		Candida albicans	*Other*	*Nil*
16 weeks	250	29* (11 6 per cent)	15	206
28 weeks	232	36* (15 5 per cent)	16	180
36 weeks	226	35 (15 5 per cent)	10	181
Post-natal	194	6 (3 1 per cent)	3	185

* One patient with thrush

TABLE 21

CANDIDA ALBICANS ISOLATIONS IN PATIENTS CULTURED ON ALL THREE OCCASIONS (16, 28 AND 36 WEEKS)

16 weeks	*28 weeks*	*36 weeks*
27+	21+ (4 treated)	18+ (3 treated)
		3− (2 treated)
	6− (2 treated)	1+
		5−
190−	12+	10+
		2−
	178− (1 treated)	5+
		173− (1 treated)
Totals 27+	33+	34+
190−	184−	183−
217	217	217

\+ = *C albicans* grown
− = *C albicans* not grown

Culture statistics.—In the previous survey of non-pregnant subjects, yeasts were cultured from 57 of 500 (11 per cent) of them, 38 subjects (7 per cent) harbouring *C albicans* and the remainder at least 5 other yeast species Six cases of clinically recognizable thrush were recorded, and smears and cultures from these patients

were all positive for *C. albicans*. Patients with severe pruritus, vulvitis, vaginitis, or profuse and curdy discharge harboured *C. albicans* significantly more often than those without these symptoms and signs

Of the 250 pregnant subjects examined in this later survey, yeasts were cultured from 75 patients (30 per cent) at some time or another during their pregnancy; 50 (20 per cent) of these had *C. albicans* in their secretions (Table 19) Two patients developed frank thrush The incidence of *C albicans* did not differ significantly at 16 weeks (11 6 per cent) from that at 28 or 36 weeks (15·5 per cent), but it was reduced at the post-natal examination (3 1 per cent) (Table 20) The incidence of other yeasts in the vaginal flora was similarly relatively unaffected by the stage of pregnancy, though the post-natal reduction is not as marked as for *C. albicans*. While the likelihood of obtaining a positive culture of *C albicans* from this group remained at the same level throughout pregnancy there was some interchange of status in individual patients (Table 21). Of 217 patients followed to 36 weeks, only 18 (8·3 per cent) were positive for *C albicans* on all three occasions, that is, at 16, 28 and 36 weeks, and 173 (79·7 per cent) were negative on all occasions Of the remaining 26 patients (12 per cent of the total), 12 were culture positive on one occasion and 14 on two occasions So few patients received specific antifungal treatment that it is considered unlikely that such treatment significantly influenced conclusions Parity did not effect either the incidence of *C albicans* or of other yeasts in the vaginal secretions

C albicans was grown from all patients with severe pruritus, and for severe and moderate pruritus cases taken together the incidence rates at 16, 28 and 36 weeks were 35, 58 and 42 per cent respectively, compared with 7, 10 and 12 per cent for comparable examinations in patients without or with only mild pruritus (Table 22) Such differences are statistically significant The incidence of yeasts other than *C albicans* was lower, not higher, in patients with pruritus, but the differences are not significant Similarly, *C albicans* occurred with greater frequency (33 per cent compared with 9 per cent) in the patients with severe or moderate vaginitis as assessed at 16 weeks (Table 23), but the occurrence of this yeast was not positively related to the presence of cervicitis (12 3 per cent in patients with cervicitis compared with 11 4 per cent in those without) (Table 24) The incidence of other yeasts did not differ significantly in these groups Comparable to what was found for vaginitis, the incidence of *C albicans* in cases of severe or moderate vulvitis was 24, 25 and 24 per cent at the 3 examination times compared with 10, 12 and 11 per cent in cases without or with only mild vulvitis

TABLE 22
CULTURE RESULTS IN PATIENTS WITH PRURITUS VULVAE AT DIFFERENT STAGES OF PREGNANCY

	Pruritus	*Number of patients*	*Yeasts cultured*		
			Candida albicans	*Other*	*Nil*
16 weeks	Severe and moderate	40	14 (35 per cent)	1	25
	Mild or nil	210	15 (7 per cent)	14	181
	Totals	250	29	15	206
28 weeks	Severe and moderate	26	15 (58 per cent)	0	11
	Mild or nil	206	21 (10 per cent)	16	169
	Totals	232	36	16	180
36 weeks	Severe and moderate	26	11 (42 per cent)	0	15
	Mild or nil	200	24 (12 per cent)	10	166
	Totals	226	35	10	181

TABLE 23
CULTURE RESULTS IN PATIENTS WITH VAGINITIS AT 16 WEEKS

Vaginitis	*Number of patients*	*Yeasts cultured*		
		Candida albicans	*Other*	*Nil*
Severe and moderate	30	10 (33 per cent)	0	20
Mild or nil	220	19 (9 per cent)	15	186
Totals	250	29	15	206

TABLE 24
CULTURE RESULTS IN PATIENTS WITH CERVICITIS AT 16 WEEKS

Cervicitis	*Number of patients*	*Yeasts cultured*		
		Candida albicans	*Other*	*Nil*
Present	65	8 (12 per cent)	5	52
Absent	185	21 (11 per cent)	10	154
Totals	250	29	15	206

TABLE 25

CULTURE RESULTS IN PATIENTS WITH VULVITIS AT DIFFERENT STAGES OF PREGNANCY

	Vulvitis	*Number of patients*	*Yeasts cultured*		
			Candida albicans	*Other*	*Nil*
16 weeks	Severe and moderate	33	8 (24 per cent)	1	24
	Mild or nil	217	21 (10 per cent)	14	182
	Totals	250	29	15	206
28 weeks	Severe and moderate	63	16 (25 per cent)	5	42
	Mild or nil	169	20 (12 per cent)	11	138
	Totals	232	36	16	180
36 weeks	Severe and moderate	76	19 (25 per cent)	5	52
	Mild or nil	*150*	*16 (11 per cent)*	*5*	*129*
	Totals	226	35	10	181

(Table 25); these differences are again significant, but are not so for other yeasts. In patients with curdy vaginal discharge the incidence rates of *C albicans* at the 3 stages were 31, 59 and 38 per cent compared with 9, 12 and 13 per cent without this type of discharge In patients with profuse vaginal discharge the incidence of *C albicans at the 3 stages were 11, 16 and 14 per cent compared* with 12, 15 and 16 per cent for those without profuse discharge (Table 26) These rates could hardly be closer and this deviation from the conclusions reached in the survey of non-pregnant patients might be explained by excessive secretions during pregnancy masking any differences which may be associated with infection

The number of patients found to have glycosuria was too small to justify valid conclusions being made upon the incidence of yeasts, in this group *C albicans* was present in 33, 12 and 24 per cent of these few patients at the 3 examination times compared with 11, 16 and 15 per cent in subjects without glycosuria

Mouth swabs from babies born of the first 100 patients were taken within 48 hours of birth, and the remainder between the sixth and thirteenth days Of 201 mouth cultures, only 1 was positive by culture for *C albicans*, vaginal cultures from the mother of this baby were also positive at the sixteenth, twenty-eighth and thirty-sixth weeks but were negative post-natally

TABLE 26

CULTURE RESULTS IN PATIENTS WITH PROFUSE VAGINAL DISCHARGE AT DIFFERENT STAGES OF PREGNANCY

	Discharge	*Number of patients*	*Yeasts cultured*		
			Candida albicans	*Others*	*Nil*
16 weeks	Profuse	80	9 (11 per cent)	4	67
	Not profuse	170	20 (12 per cent)	11	139
	Totals	250	29	15	206
28 weeks	Profuse	69	11 (16 per cent)	4	54
	Not profuse	163	25 (15 per cent)	12	126
	Totals	232	36	16	180
36 weeks	Profuse	70	10 (14 per cent)	2	58
	Not profuse	156	25 (16 per cent)	8	123
	Totals	226	35	10	181

Conclusions.—There seems little doubt that pregnancy creates conditions of increased vaginal susceptibility to colonization by *C. albicans* in particular and by other yeasts in general The two surveys in which non-pregnant and pregnant states were compared were, however, carried out in different populations and over different periods in time The incidence of *C albicans* in the vagina did not increase significantly as pregnancy advanced but a dramatic fall in incidence occurred post-partum Mouth cultures from babies born of these subjects, including those repeatedly shown to harbour *C albicans* in the vagina during pregnancy, were with one exception negative for fungi on culture There is no reason to believe that the very low incidence of *C albicans* isolated from vaginal swabs taken post-partum, and also from babies' mouth swabs, was due to the delay in culture resulting from sending them through the post

The same positive relationship between the incidence of *C albicans* and severe and moderate pruritus and vulvitis, and also curdy discharge, was noted for these pregnant patients as was found in the non-pregnant group, again no such relationship existed for cervicitis The one contradiction occurred in connexion with profuse vaginal discharge in the previous paper a significant (though only slight) increase in incidence of *C albicans* occurred in patients with this sign, but the observation was not confirmed in this more recent investigation in pregnancy The possible reason for this has been given.

ACKNOWLEDGEMENT

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SKIN MONILIASIS IN INFANTS

J. P. BOUND

SKIN MONILIASIS is common in infancy, but is usually confined to the napkin area. In a recent investigation it was found that at least 3 per cent of the babies born in a London obstetric hospital had been affected by the age of 6 months. Other body flexures may be involved, and rarely the rash becomes widespread. Infection of the nails also seems to be rare in infancy.

The following description of monilial napkin rashes is based on the appearance in 42 cases found in 1 year among infants born in the Obstetric Hospital or attending the Infant Welfare Department of University College Hospital, London In all cases cultures were made for the presence of *Candida albicans* in swabs from the lesions, it being shown that it could not be grown from swabs of other types of napkin rash or from the napkin area of healthy babies. The yeast was identified as *C albicans* in 39 of the 42 cases. In infants with oral thrush but no napkin rash *Candida* could be grown from the perianal skin in about half the cases, but it should be noted that less than one-third of the babies with a monilial napkin rash had oral lesions

The appearance of a monilial napkin rash is typical. The common sites to be affected are the buttocks and inner thighs, most cases have lesions in both areas The genitalia and lower abdomen may also be involved and sometimes these parts, or the inner thighs, are affected while the buttocks remain clear The early lesions consist of dull red areas which often coalesce, except peripherally Desquamation often occurs, usually after the formation of flat, superficial vesicles In isolated lesions the peripheral part of the thin white epithelium frequently remains behind as a collar, producing an appearance characteristic of skin moniliasis Sometimes the condition begins as an intertriginous lesion of the groins, but the presence of white, macerated epithelium is diagnostic This is the usual form taken by the rash in other body flexures, but occasionally the dry, desquamating, dull red rash is seen at these sites

Without specific treatment monilial napkin rashes usually persist for many weeks. Not uncommonly the true nature of the eruption is unrecognized and it is attributed to a low standard of maternal care

It will be clear from the foregoing description that monilial infection is readily distinguishable from two other common napkin rashes, namely, excoriated buttocks and ammonia dermatitis. Seborrhoeic dermatitis in the napkin area may resemble moniliasis, but desquamating white epithelium is not seen and there are typical lesions elsewhere. However, seborrhoeic dermatitis seems to predispose to monilial infection and it is advisable to look for the organism in all cases with involvement of the buttocks

In the series of cases already mentioned, two factors that might be expected to play an important part in the aetiology of monilial napkin rashes were investigated. These were maternal thrush vaginitis and previous antibiotic therapy in the infants

THRUSH VAGINITIS

It was found that there was a significantly increased incidence of thrush vaginitis in the mothers of babies born in the hospital and who subsequently developed a monilial napkin rash compared with a sample of mothers delivered in the same year. Twenty-seven per *cent of mothers of babies with napkin rash had thrush vaginitis, and others may have had an asymptomatic infection. These findings,* together with the fact that nearly half the infants developed rashes at home and the fact that less than one-third of them had oral thrush, suggested that the infection was often transferred to the infant's skin by the mother's hands when the napkin was changed Monilial rashes occur chiefly in the first 6 weeks of life (in 85 per cent of cases) when the skin is sensitive and liable to irritation, particularly in the napkin area, and therefore likely to present conditions which permit *C albicans* to thrive.

PREVIOUS ANTIBIOTIC THERAPY

The incidence of previous antibiotic therapy was not significantly greater in babies with monilial napkin rash compared with other babies born in the hospital, and only in 3 did rashes appear within 1 week of such therapy It appears, therefore, that antibiotic therapy plays little part in the aetiology, although it may be a precipitating factor in a few cases

TREATMEAT

Treatment by painting the skin with 1 per cent aqueous solution of gentian violet is satisfactory but has obvious disadvantages The majority of cases were treated by the application of 0 1 per cent aqueous solution of merthiolate followed by a protective paste of titanium dioxide each time the napkin was changed This method

does not stain the infant's clothes, and mothers carried it out effectively. The rash cleared up in 1 to 6 weeks, the average time being about 2 weeks. Recurrences were seen in 16 per cent of cases from 2 to 10 weeks after the first rash had cleared. They quickly responded to further treatment. One infant had two more recurrences before he was a year old. Nystatin ointment was used in a few cases; it cleared the rash effectively, but experience was too small to say if it has any advantage over the other method of treatment

Skin moniliasis is common in infancy and should always be considered in the diagnosis of napkin rashes. Recognition is usually easy, but less than one-third have associated oral thrush Treatment is simple and effective. Maternal thrush vaginitis is an important aetiological factor

INFANTILE MONILIASIS

MORBID ANATOMY

MARTIN BODIAN

CANDIDA INFECTION IN THE NEWBORN

In a recent review from New York (Taschdjian and Kozinn,[1] 1957) nearly 4 per cent of over 2,000 newborn infants had *Candida* (*Monilia*) in their mouths and practically all of these developed clinical thrush infection *Candida* was found invariably in the stools of those who carried the organism in their mouths, and more than 50 per cent of these cases had cutaneous moniliasis in the perianal and diaper area The autopsy findings of 7 cases investigated at The Hospital for Sick Children, Great Ormond Street, London, will be described to illustrate various aspects of *Candida* infections In the newborn this infection is often associated with persistent vomiting which presumably leads to denudation of oesophageal mucosa The following 4 cases, including 2 in premature infants, are examples

Case 1 was born after a gestation period of 32½ weeks and weighed 2 pounds 11 ounces. This was a possible case of Hirschsprung's disease presenting with symptoms of intestinal obstruction Although a colostomy was carried out, the child did not improve and developed jaundice and bronchopneumonia, and died At post-mortem examination there was extensive oesophageal and pharyngeal ulceration and bronchopneumonia, but no evidence of Hirschsprung's disease The oesophagus was the seat of considerable infection with *Candida albicans* (Fig 67).

Case 2 presented with high jejunal atresia for which duodeno-jejunostomy was performed Jaundice, subarachnoid haemorrhage and kernicterus of prematurity followed, and finally death from bronchopneumonia At autopsy, *C albicans* was present in areas of mucosal ulceration of the posterior pharyngeal wall, oesophagus and stomach It was also present in both lungs

Case 3 had oesophageal atresia with a tracheo-oesophageal fistula as well as rectal agenesis and a high recto-urethral fistula Treatment consisted in ligation of both fistulae, colostomy and oesophageal anastomosis This anastomosis broke down, resulting in mediastinal infection and aspiration pneumonia Yeasts and pseudomycelium of *C albicans* were present in bronchioles (Fig 68) and alveoli

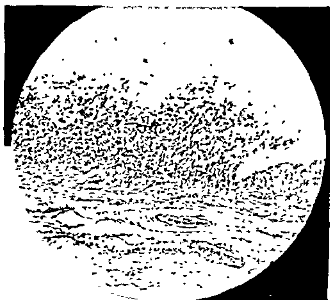


FIG 67 —*Candida* infection of oesophagus (Case 1)



FIG 68 —Section of lung showing *Candida* infection of bronchioles (Case 3) (× 225 Stained periodic acid Schiff and haematoxylin)

Case 4 presented with oral thrush infection and vomiting, and developed bronchopneumonia and died. At autopsy he was found to have bilateral cystic disease of the kidneys. *Candida* infection was found only in the mouth.

None of these newborn infants died as a result of *Candida* infection. Rather does invasion by this organism appear to be an index of debility particularly associated with severe congenital malformations and/or prematurity. All the infants described presented with severe persistent vomiting

CANDIDA INFECTION IN OLDER CHILDREN

[illegible]

of the pancreas The former cases were particularly those undergoing treatment with anti-metabolites and/or steroids, and the latter those who had received repeated courses of antibiotics or prolonged maintenance treatment with these drugs This infection was also associated with nephrosis

In leukaemia there is an increased susceptibility to infections, including moniliasis. Antibiotics tend to prolong the life of these debilitated patients but may also increase the opportunity for invasion by *C. albicans* Extensive ulceration of oropharynx and gastro-intestinal tract and a pseudo-membranous enterocolitis are commonly observed The extensive membranes are formed of epithelial debris, large colonies of bacteria and sometimes of *Candida* hyphae and spores These findings are particularly common in cases treated with cortisone

Winter and Foley[2] (1956) reported from the Children's Cancer Research Foundation in Boston that 41 per cent of children with untreated leukaemia and 59 per cent of cases of leukaemia treated with antibiotics and cortisone harboured *Candida*, compared with 15 per cent of a control series of approximately 1,000 children In cases of fibrocystic disease of the pancreas, the incidence of *Candida* infection was 29 per cent compared with 15 per cent in the controls There was an incidence of 31 per cent in nephrotic syndrome and of 60 per cent in diabetes mellitus

Case 5 is a case of leukaemia with *Candida* infection in a child aged 7 years with a symptomatic course of 7½ months Treatment was with blood transfusions, Aminopterin and cortisone, which produced short remissions At autopsy, there was extensive leukaemic infiltration of



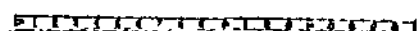


Fig 69 —Multiple gastric ulcers invaded by *Candida* in leukaemia (Case 5)





Fig 70 —Pseudomembranous colitis with *Candida* infection in leukaemia (Case 5)



Fig 71 —Section of ileum in leukaemia showing invasive *Candida* pseudomycelium and ulceration (Case 5) (× 36 6 Stained periodic acid Schiff and haematoxylin)



FIG 72.—Thrush oesophagitis (Case 6)



FIG 73 —Thrush proctitis (Case 6)



FIG 69 —Multiple gastric ulcers invaded by *Candida* in leukaemia (Case 5)



FIG 70 —*Pseudomembranous colitis* with *Candida* infection in leukaemia (Case 5)



FIG 71 —Section of ileum in leukaemia showing invasive *Candida* pseudomycelium and ulceration (Case 5) (× 36 6 Stained periodic acid Schiff and haematoxylin)

INFANTILE MONILIASIS

PATHOLOGY

I. A B CATHIE

Candida albicans cannot be considered as a complete pathogen in children, the normal subject being insusceptible to infection by this yeast. Rather should it be thought of as a facultative pathogen requiring the assistance of some other abnormality or debilitating condition to establish itself as of more than nuisance value.

Many cases of clinical thrush never come to the notice of the clinical pathologist, being either ignored or treated on their merits without bacteriological confirmation Furthermore, many cases of severe thrush are severe only because of the severity of the precipitating illness which permits moniliasis to become established, so that the major investigative work tends to be focused on the primary condition and not on the thrush In considering the laboratory records on moniliasis from The Hospital for Sick Children, London, it is important to bear in mind these facts, and also that cases come to this hospital from a wide area, intake from local population and welfare clinics being minimal

The strains of *Candida* isolated in 1956 at this hospital divide themselves automatically into those from the mouth, including cases of oral thrush, and those from other parts of the body

ORAL MONILIASIS

C albicans was recorded in the mouth swabs of 29 babies, 17 of whom suffered from gastro-enteritis This does not represent the incidence of mouth infection with this yeast, the 29 cases being those in whom the mouth lesion was sufficiently severe to call for investigation Of the 17 cases of gastro-enteritis, oral moniliasis seems not to have been present on admission in any case but to have developed after a few days in hospital These patients were all admitted to hospital in the acute phase of their illness with a history of one or two days duration only It appears that the debilitating effect of acute infection necessary to render the baby a suitable subject for thrush does not take place suddenly, but needs some days for the appropriate conditions to be developed Four of these babies had *C albicans* in their stools but the possibility of monilial enterocolitis

lymph nodes, liver, and kidneys. Monilial infection of areas of ulcerative pharyngo-oesophagitis, gastric ulceration (Fig. 69) and membranous enterocolitis, especially in the ileo-caecal region, was present (Figs. 70 and 71).

Case 6 is an example of bronchiectasis with *Candida* infection. This child aged 19 months had been well until pertussis infection and bronchopneumonia occurred at 6 months. Measles followed at 9 months and evidence of bronchiectasis was present from the age of 11 months. Thrush was persistently present in the mouth from 7½ months Weight deteriorated from 24 pounds at 6 months to 15 pounds at 15 months. There was an extension of thrush to the uvula and epiglottis at this time and laryngeal stridor developed. Tracheotomy was performed but death followed. Post-mortem examination showed moniliasis with ulceration of the mouth, pharynx, larynx, oesophagus (Fig 72), vulva, rectum (Fig. 73) and anus.

Case 7 exemplifies nephrosis with *Candida* infection. This child of 3 years of age suffered from a throat infection at 17 months and 2 weeks later developed a nephrotic syndrome. Oedema, albuminuria and hyper-cholesterolaemia were present. There were remissions with ACTH and later with cortisone. Urinary infection followed and then a rise in blood pressure and pyrexia prior to death. Autopsy showed nephrosis, acute pancreatitis of an annular pancreas and acute duodenitis Moniliasis of the oesophagus and stomach was found.

In none of these instances was infection by *C. albicans* thought to have contributed materially to death. It was considered as an index of susceptibility to infection in a debilitated child produced by the leukaemic process, by the presence of abnormally viscid mucous secretions in a medium of high salt concentration in fibrocystic disease of the pancreas, and by other factors in other diseases We have seen no instance of *Candida* septicaemia of the kind reported in the literature producing granulomatous lesions in various viscera, notably the kidneys and meninges

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seem to suffer from the defect that they are either toxic or inactive when given systemically, and when administered topically suffer from the drawback that they are difficult to maintain *in situ* long enough to exert their fungicidal action Local treatment with gentian violet continues to be used and thiomersalate, effective in high dilutions *in vitro*, has frequently been employed topically. Encouraging results have been obtained with pentamidine isethionate administered intravenously, and strains of *C albicans* so far encountered have all been sensitive to 1·5 microgram per millilitre or less of this agent; in few cases only, however, has moniliasis called for such treatment Usually, these infections resolve as the underlying disease process is corrected by general measures

comparable to that seen in the adult was not entertained. It is a matter for speculation whether the development of thrush in these cases of gastro-enteritis was, as is suggested, consequent upon the debilitating effect of the disease, or whether antibiotic therapy was responsible by removing bacterial competitors from the flora of the alimentary tract. The moniliasis, occurring as it did after the acute phase of gastro-enteritis, was more an obstacle to rapid recovery than a cause of clinical deterioration.

NON-ORAL MONILIASIS

C albicans was found in a variety of situations other than the mouth, including sputa, a mastoid cavity and the skin; there was little experience of thrush of the napkin area but 5 cases of peri-anal dermatitis were associated with *C. albicans*. Compared with adult findings it is of interest that *C albicans* was not isolated from the 155 vaginal swabs investigated during 1956. One fatal case with generalized moniliasis was encountered, the yeast being grown from the lungs, pleura, peritoneum, gut and from the blood; this occurred in a baby with septicaemia following pneumonia and lung abscess. In a further fatal case, *C albicans* was cultured from a post-mortem lung swab from a girl with multiple congenital anomalies, *Shigella sonnei* was also isolated but its origin was never elucidated The various pathological conditions occurring in this patient included rectal agenesis, hydrocephalus, ulcerative colitis, Hirschsprung's disease, agammaglobulinaemia, congenital cystic kidney and nephrosis, any of which would produce a degree of debility sufficient to predispose to moniliasis No case of moniliasis in this series was primary in nature, or the only disease process present

The major clinical problem in another instance was generalized *Trichophyton* infection One child undoubtedly died from pulmonary moniliasis, she was an old case of bronchiectasis in whom antibiotic therapy had removed every susceptible pathogen and commensal *C albicans* heavily infected the whole respiratory system and the bronchiectatic cavities, and no treatment was of avail

TREATMENT

Only seldom does thrush call for vigorous specific therapy, in severe infection only too often is the site of the moniliasis inaccessible to

the antibacterial antibiotics are of value in the treatment of moniliasis as judged by laboratory experience Most of the new fungicides

INFECTIONS DUE TO AEROBIC ACTINOMYCETES

In Table 27 are listed the main infections due to actinomy
Some of these are systemic infections like actinomycosis, due
israelii[13, 14], and nocardiosis, due to *Nocardia asteroides*. O
other hand, *some infections are subcutaneous*, the most impc
of these being the so-called actinomycetomas. Finally,
diseases are cutaneous, such as erythrasma, due to *N. minutis*
and trichonocardiosis axillaris due to *N. tenuis*

PATHOGENIC AEROBIC ACTINOMYCETES

Five species of aerobic actinomycetes are important from a cl
point of view, 2 belonging to the genus *Nocardia* (*N. asteroide*
N brasiliensis) and 3 to the genus *Streptomyces* (*S. madure*
pelletieri, and *S somaliensis*)

N asteroides is the main causative agent of systemic aerobic ac
mycosis This may involve numerous organs, the lungs bein
most frequent Pulmonary nocardiosis may simulate tuberc
and confusion may further arise because *N asteroides* is partially
fast and frequently bacillary in form in specimens sent for diag
Though disease may apparently be limited to organs other tha
lungs, it is most likely that primary disease is pulmonary in n
Infection of brain and meninges may give rise to symptom
signs simulating a brain tumour[9, 13] Systemic nocardio
assumed, perhaps incorrectly, to be a rare condition

N asteroides may also cause subcutaneous disease of "mycet
type This is generally an infection of the foot or the leg, some
of the hand or the arm, which follows a traumatic introductio
the dermis of a pathogenic fungus (causing maduromycetom
actinomycete (causing actinomycetoma), less frequently the
may be involved Actinomycetoma tends to be a ch
slowly progressive infection characterized by multiple subcuta
abscesses discharging pus containing white or pigmented gra
through numerous fistulae Bones are sometimes involved res
in progressive osteolytic disease Any of the 5 species ment
above may cause this type of infection Instances of system
semination of disease following subcutaneous infection have
reported Mycetomas are fairly frequent in all arid, tropic
subtropical areas[1, 2]

INFECTIONS DUE TO AEROBIC ACTINOMYCETES

F. MARIAT

ACTINOMYCETES are bacteria producing a filamentous growth of branching hyphae. Despite this fact, the pathogenic actinomycetes and the diseases they cause have received special attention by medical mycologists, so they may properly receive consideration in a symposium on fungous infections Most of the pathogenic actinomycetes occur in Nature and belong to the soil saprophytic flora Only the anaerobic species, *Actinomyces israeli*, is a normal inhabitant of the mouth and an obligate parasite

The filaments of actinomycetes do not exceed 1 micron in diameter, they develop branches which come off at right-angles Sometimes the hyphae break up into bacillary or coccoid elements *In vivo*, the filaments may be free in the tissues or become aggregated into granules which may or may not be surrounded by clubs *In vitro*, most of the actinomycetes can grow aerobically, but *A israeli* is an anaerobe or micro-aerophile

On adequate media, the aerobic actinomycetes grow as white or pigmented colonies, the surface of which is more or less wrinkled, glossy, downy or chalky In culture some pathogenic strains produce filaments which become fragmented into bacillary or coccoid forms (genus *Nocardia*), some species of this genus are partially acid-fast Other strains of actinomycetes (genus *Streptomyces*) develop a mycelium which does not fragment but which, under certain conditions, produces chains of conidia and sometimes spirals

TABLE 27
MAIN INFECTIONS DUE TO ACTINOMYCETES

SYSTEMIC	Actinomycosis	*A israeli*	Anaerobic culture
	Nocardiosis	*N asteroides* *N brasiliensis*	Aerobic culture
SUBCUTANEOUS	Mycetoma	*S somaliensis* *S pelletieri* *S madurae*	
CUTANEOUS	Erythrasma Trichonocardiosis	*N minutissima* *N tenuis*	Culture uncertain (diagnosis by direct examination only)

TABLE 29

CHARACTERISTICS OF CULTURES OF THE MOST IMPORTANT AEROBIC ACTINOMYCETES CAUSING NOCARDIOSIS AND ACTINOMYCETOMA

	Pa fas	Fr of				dextrose agar	Ex pat for
N asteroides	+	+	Yellowish-white to orange	Rapid 3–8 days	30–37 C	Smooth, soft, glabrous, irregularly folded (Figs 74b and 75b)	+
N. brasiliensis	+	± → +	Yellowish-ochrous-red	Rapid 3–8 days	30 C approximately	Hard colonies, folded, and often with chalky surface (Fig. 76b)	+ or −
S somaliensis	−	−	Whitish, then brown or blackish	Rapid	30°C	Flat, wrinkled colonies, glossy and later dull (Fig 77b)	−
S pelletieri	−	−	Pink-coral red	Slow 8–30 days	37°C	Heaped, glabrous, irregular, hard colonies (Fig 78b)	−
S madurae	−	−	Whitish to red	Slow 8–20 days	37°C	Usually deeply folded with moist glabrous and waxy surface (Fig 79b)	−

TABLE 30

Species	*Utilization of*												*Hydrolysis of*			
	Urea	$(NH_4)_2SO_4$	$(NH_4)_2HPO_4$	NH_4NO_3	KNO_3	Xylose	Levulose	Galactose	Maltose	Starch	Mannitol	Paraffin	Gelatin	Serum	Ovalbumin	Starch
N asteroides	+	+	+	+	+	−	+	−	−	−		+	−	−	−	−
N brasiliensis	+	+	+	+	+		+	+			+	+	+			−
S somaliensis	−	−	−	−	−	−		−	+	−	−	−	+	+	+	−
S pelletieri	+	−	+	−	−	−		−	−	−	−	−	+	+	+	−
S madurae	+		+			+	+			+	+	−		+	−	+

most important single investigation of these cases and one which, unfortunately, is often neglected The most suitable media for *primary isolation are nutrient agar, Sabouraud's dextrose agar*, potato glycerol agar and Loewenstein-Jensen egg medium

The properties of the aerobic actinomycetes causing human disease are summarized in Tables 28, 29 and 30 These concern such features as colonial morphology, staining properties, enzymatic activity, *utilization of carbon and nitrogen compounds*[8, 11, 12] Figures 74–79 illustrate the characteristics of these organisms in tissues and in culture.

TABLE 28

MORPHOLOGICAL CHARACTERISTICS OF THE "GRAINS" OF ACTINOMYCETOMA IN PUS OR OTHER PATHOLOGICAL MATERIAL, AND GEOGRAPHIC DISTRIBUTION OF INFECTIONS

Species	*Characteristics of the "grains" of actinomycetomas*				*Geographic distribution of infections*
	Colour of "grains" in pus	*Clubs*	*Staining by haematoxylin*	*Morphology*	
N asteroides	Yellowish-white	Rare	–	Consists of net-work of filaments, sometimes swollen at ends and frequently fragmented (Figs 74a and 75a)	America, Europe, Asia, Africa
N. brasiliensis	Yellowish	Rare	–	Irregular and variable in size made up of lobules (Fig 76a)	South and Central America, sometimes elsewhere
S somaliensis	Yellow	Absent	–	Regular (approximately *1 mm diameter*) with smooth edges Composed of filaments embedded in hard cement, slits frequently seen in sections (Fig 77a)	Sudan, Somaliland, Ethiopia, Senegal, Nigeria
S pelletieri	Red	Absent	+	Small (approximately 500 microns) and numerous Smooth or denticulate edges Main grain frequently fragmented in 2 or 3 secondary granules with geometrical shapes (Fig 78a)	Senegal, Nigeria, Brazil, Mexico, South Africa, India, Sudan
S madurae	Yellowish-white to reddish	Frequent	+	Large "grains" several millimetres in diameter with *lobulate edges* Clubs usually long, narrow, and sometimes branched (Fig 79a)	India, Asia, Africa, Europe, America



FIG 76—(a) Section of a mycetoma due to *N brasiliensis* (b) Culture of *N brasiliensis*

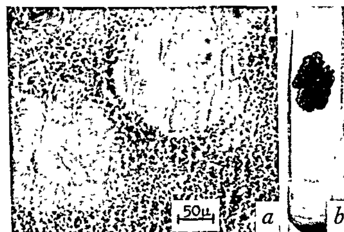


FIG 77—(a) Section through a "grain" in infection due to *S somaliensis* (b) Black colony of *S somaliensis*



FIG 74.—(a) Section showing a granule of *N. asteroides* (c) Culture of *N asteroides*



FIG 75.—(a) Section of brain tissue from a fatal case of cerebral nocardiosis Numerous delicate, branching, fragmented filaments of *N asteroides* present (b) Culture

TREATMENT

The treatment of these infections is often difficult and surgery often becomes necessary; early diagnosis gives the best prognosis. Sulphonamides are frequently inhibitory to pathogenic species of *Nocardia* and *Streptomyces*. The few successfully treated cases of pulmonary or cerebral nocardiosis received sulphadiazine alone or in association with streptomycin.

When there is an extensive involvement of deep tissues, a mycetoma may have to be removed by amputation of an extremity. In such cases, sulphonamides should be given both pre-operatively and post-operatively, together with antibacterial agents for preventing secondary infection.

Mexican authors report good results in treating mycetomas due to *N. brasiliensis* with 4·4'-diaminodiphenyl sulphone (DDS) as in leprosy[3, 4, 5]. *In vitro*, *S. madurae* seems more susceptible than *N. brasiliensis* to this drug (10 out of 12 strains tested were susceptible to 15 micrograms per millilitre and all of the same 12 strains were susceptible to 30 micrograms per millilitre after a 3 weeks' incubation period)[7]. It would seem possible, therefore, that this sulphone might also be of value in the treatment of mycetomas due to *S. madurae*.

The infrequency with which diseases due to aerobic actinomycetes are diagnosed may be due in part to their being poorly known and therefore infrequently brought to mind in differential diagnosis. As for the true mycoses, increased frequency of identification of diseases is often the result of activity of groups of workers particularly interested in them. A large number of aerobic actinomyces species have been falsely described as being pathogenic, a fact which may be explained by the relative difficulty of cultural diagnosis owing to their great variability and to the ease with which they may be confused with the more frequently occurring contaminant species.

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FIG 78 —(a) Section of "grain" in a mycetoma due to *S pelletieri* (b) Culture of *S pelletieri*



FIG 79 —(a) Section of a mycetoma due to *S madurae* (b) Culture of *S madurae*

BRONCHO-PULMONARY ASPERGILLOSIS

K. F. W HINSON

IN the examples of broncho-pulmonary aspergillosis which will be discussed, *Aspergillus fumigatus* was the responsible fungus and was identified on cultural and morphological grounds. Other species have been described as causing the disease such as *A niger*, *A. versicolor*, *A nidulans* and *A. flavus* but there is little doubt that in Britain the infecting fungus is usually *A fumigatus* Since this is such a common laboratory contaminant, it is important to exclude this possibility especially if the diagnosis is based on cultural findings Single isolations from sputum should not be stressed and the fungus should be demonstrated repeatedly The specimen should be collected in a sterile glass jar, not waxed paper cartons, and inoculated on blood agar medium which has been spread with 1.1,000 streptomycin in order to inhibit commensal bacteria, or on Sabouraud's medium Incubation at 37° C should continue for 48 hours.

It has been recognized for many years that this fungus occurs in infarcted areas of the lungs and it is probable that *A. fumigatus* makes a saprophytic invasion of infarcts in lungs affected by bronchiectasis or pneumoconiosis An example occurred in a man aged 31 years, a french polisher, who had suffered for many years from bilateral bronchiectasis Following an intravenous injection he developed thrombosis of an arm vein and died 3 days later from pulmonary embolism Fig 80 illustrates the proliferation of the mycelium within alveolar spaces, alveolar walls and thrombosed vessels The fungus forms its fruiting heads within an infarct (Fig 81) and the species may be identified with ease

Location and appearance.—The commonest manifestation of this fungus disease is the mycetoma (Fig 82) Such cases usually give a long history of repeated small haemoptyses over months or years Radiologically there is a cavity with a solid content which may be mobile With one exception the mycetomas seen personally have been in the upper lobe the exception was situated in the apical segment of the lower lobe The wall of the cavity is almost always lined by epithelium of the respiratory type (Fig 83) and the content is a moist brown crumbling mass of mycelium Some fibrin is present but there is little inflammatory exudation The mycelium

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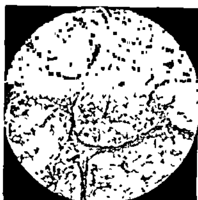


FIG 80 —Aspergillus mycelium permeating alveolar walls and a thrombosed vessel in a pulmonary infarct (Masson, ×112)



FIG 81 —*Aspergillus fumigatus* within lung infarct. hyphae and sporing structures (Masson, ×200) (*By courtesy of the Editor, Thorax*.)



FIG 82 —Excised segment showing a bronchiectatic cavity 1 inch in diameter containing a mycetoma There are 2 smaller cysts above (*By courtesy of the Editor, Thorax*)

is strikingly outlined by silver impregnation methods (Fig 84) It is unusual for sporing structures to be present in the mycelial mass, but they may occasionally be found in freshly resected specimens

Mycetomas are associated with other diseases causing slowly progressive pulmonary fibrosis Several examples in cases of asbestosis have been met with in personal experience The relationship is obscure as the fibrosis and bronchiectasis in this condition are

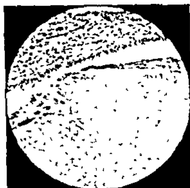


FIG 83 —Section of cavity wall and mycelial mass of an aspergillus mycetoma (Haematoxylin and eosin, ×150) *(By courtesy of the Editor, Thorax)*

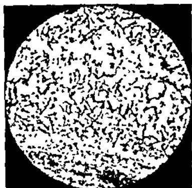


FIG 84 —Silver impregnation preparation of section shown in Fig 83 (×52)



FIG. 85 —Mycetomas in the upper zone of a fibrotic lung following sarcoidosis.

most severe in the lower lobes, yet in each instance the mycetoma was in the upper lobe In those cases of sarcoidosis which proceed to fibrosis, mycetomas may develop Fig 85 shows a whole lung section with 2 such lesions in the upper lobe Sarcoidosis had been diagnosed on lymph-node biopsy 8 years before death Cavities

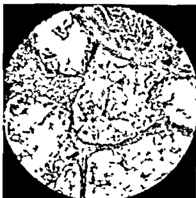


FIG 80 —Aspergillus mycelium permeating alveolar walls and a thrombosed vessel in a pulmonary infarct (Masson, ×112)



FIG 81 —*Aspergillus fumigatus* within lung infarct hyphae and sporing structures (Masson, ×200) (*By courtesy of the Editor, Thorax*)



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be slightly bloodstained, and often wheezing which may be severe. There is a blood eosinophilia of 1,000 or more per cubic millimetr Radiographs show areas of collapse and consolidation.

With each febrile episode the changes may occur in different lobe or on different sides, or even from side to side and back again. Tw of these cases were bronchoscoped during such an attack and bronchus was seen to be blocked by a tumour-like mass. Biops of this showed mucus and fibrin which contained sparse myceli elements (Figs 87 and 88). Following this observation sputu

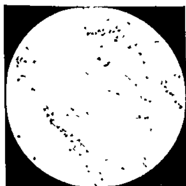


Fig 87 —Bronchial biopsy Inspissated mucus separated by layers of fibrin and some inflammatory cells (×52)

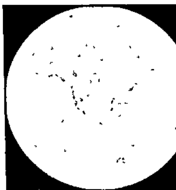


Fig 88 —Silver impregnation pr paration of section shown in Fi 87 (×112)

cultures were made and the fungus isolated repeatedly Macr scopically, the sputum from such patients will often contain sma brownish flecks which should be selected for inoculation Occasio ally these flecks will be aggregated into plugs which histological resemble the bronchial biopsy Once they are expectorated clinic improvement usually follows

The course of this type of illness is prolonged for several year One patient died in status asthmaticus and his bronchi were four to be occluded by this same type of allergic exudate

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which remain patent after the bacteriologically successful treatment of proven tuberculosis have also been observed to fill with a mass of mycelium.

Empyemic infection.—Infection of the pleural space may follow surgical intervention. During a difficult lobectomy a mycetoma ruptured into the pleura. A broncho-pleural fistula and an empyema developed. This was infected by proteus, staphylococci and *A fumigatus* At post-mortem examination the glistening wall of the empyema space was studded with sporing colonies of the fungus (Fig 86). Further examples of mixed infective empyemata have

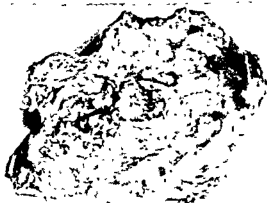


FIG 86—Colonies of *Aspergillus fumigatus* on the wall of an empyema sac

been seen following pneumonectomy In another case, a lobectomy for long-standing fibrocaseous tuberculosis, broncho-pleural fistulae and empyemata occurred Staphylococci, *Mycobacterium tuberculosis* and *A fumigatus* were present in the purulent fluids When the pleura was excised some months later it was noted that a muscle graft which had been applied to the bronchial stump at the pneumonectomy was permeated by the mycelium There was some evidence that cross-infection may have been responsible for this unfortunate occurrence

Differential manifestation.—There is yet another manifestation of aspergillosis described by Hinson, Moon and Plummer[1] in which sensitization to the fungus leads to blockage of a bronchus by an outpouring of mucus, some Charcot-Leyden crystals and some eosinophilic polymorphs In this condition there are repeated episodes of febrile illness with cough, purulent sputum which may

hyphae are in contact with the surrounding polynuclear reaction (Fig. 89). This exemplifies the pathogenic form of the fungus.

Broncho-pulmonary aspergilloma.—Extracted from its cavity through a surgical excision the substance of a broncho-pulmonary aspergilloma is brown in colour, lobulated, and usually a few centimetres in diameter. In cross-section it shows no semblance of organization though it is zonated into stripes following the contours



FIG. 89.—Diffuse pulmonary aspergillosis. Colony of aspergillus with radiating branching filaments. (Haematoxylin and eosin. ×260.)

of the surface (Fig. 90). The mycetoma is found microscopically to consist of a network of fungous filaments, and the striped appearance seen macroscopically is related to differing densities of fungous filaments. Alternating rapid and slow growth phases of the fungous mass probably induces this zonation. The aspergillus filaments have a relatively constant diameter of from 4 to 6 microns; they are septate, little branched, intermingled and much ramifying. Protoplasmic detail is almost absent[24], but the cell membrane is easily stained by dyes, particularly by haematoxylin and eosin. In some zones the filaments show hyphal swellings with little protoplasmic structure[24], the largest measuring about 30 microns in diameter, these structures are round or irregular, and are single or, rarely, grouped[24]. In the vicinity of such a zone, two or three aspergillary fructifications of

PULMONARY ASPERGILLOSIS: SOME ASPECTS OF THE PARASITIC FORMS OF ASPERGILLUS

G. SEGRETAIN

GROWTH of aspergillus in the lung was recorded about 100 years ago, and the first cases of clinical aspergillosis were described 60 years ago Renon[20] reviewed this condition in a book in 1897, and since then many papers on this subject have been published Nowadays, this infection is encountered relatively frequently in a new clinical form, "l'aspergillome bronchectasiant" or bronchial aspergilloma, which was described for the first time in 1938 by Deve[4] Recently, Monod and his colleagues pointed to its rather common occurrence[15, 17, 18] In 1956, Meyer and his colleagues[14] reviewed 21 cases of aspergilloma[2, 4, 6, 7, 9, 10, 11, 13, 15, 17, 18, 26], while in the last 2 years 12 new cases have been found[3, 5, 14, 16, 19, 21, 22, 23, 25] All of these cases have been described in the literature in Europe and the list is certainly not complete. In this form of infection, the *Aspergillus* forms a large compact body within a cavity lined by epithelium In the two other types of pulmonary aspergillosis, localization of the fungus is different In aspergillary bronchitis[8, 20], the fungus grows only on the surface of the bronchial epithelium, while in diffuse pulmonary aspergillosis[1, 12] it grows within the pulmonary tissues

Aspergillary bronchitis—Bronchial casts have been observed in the sputum in aspergillary bronchitis These casts consisted of mucus in which the fungus was growing as slender, slightly branching filaments approximating in diameter to those seen in saprophytic cultures Typical organs of fructification develop, natural aeration of the bronchi appearing to favour sporulation Instead of spreading over the surface of the bronchial mucosa the fungus may form little compact masses which are periodically expectorated These rarely exceed half a centimetre in size and are formed by entangled fungous filaments which sometimes show fructifications within the mass[24] The fungus is rarely invasive and the bronchial mucosa shows only superficial changes

Pulmonary aspergillosis.—In contrast, in pulmonary aspergillosis the fungus forms small or large colonies consisting of filaments having a general radiating form These filaments are short, twisted, frequently branched and of almost constant diameter; the peripheral

Enjalbert and her colleagues have studied this last pathological finding[5] and have noted that the lobules of an aspergilloma represent zones of active fungous growth coming into contact with a lining [illegible] absent In such areas the [illegible] contact with the cellular [illegible] Between the lobules the epithelium tends to be thickened.

In two cases of aspergilloma, some small fungous colonies have actually been observed at the periphery of the mass, round or oval in shape and formed by joining and radiating filaments The filaments at the periphery of the colonies were less abundant, frequently branched, and in contact with the surrounding polymorphonuclear leucocytes[5]. These appearances, observed by crushing the



FIG 91 —Crushed fungous mass of aspergilloma Radiating and branching filaments representing the invasive form of the fungus (Phase contrast × 520)

fungous mass (Fig 91), resemble the parasitic form of the fungus occurring in diffuse pulmonary aspergillosis, but are of rare incidence The usual appearance of the fungus in contact with cellular reaction in the lung is similar to that of the general filamentous structure of a mycetoma (Fig 92)

Exceptionally the fungus occurs elsewhere than in the lung, it has been localized to a lymph node in which the filaments of aspergillus tended to be scattered, and to show round bulbous forms within

abnormal appearance have been seen consisting of large and flattened sterigimata surrounding large vesicles[5], but these are very rarely found.

EVOLUTION OF ASPERGILLOMA

A series of chest radiographs published by Pesle[19] shows the evolution of an aspergilloma over the years It would appear that the bronchial cavity increases in dimension *pari passu* with the



Fig 90 —Section of a bronchial aspergilloma The cavity contains a fragment of the zonated fungous mass (Haematoxylin and eosin)

growth of the fungous mass Aspergillus hyphae do not, however, penetrate the modified bronchial epithelium lining the cavity Rarely, in cases of aspergilloma, the fungus may invade the pulmonary tissues and as evidence of this it has been observed that (*a*) two or more bronchi may open in the same aspergilloma cavity[4, 10], and (*b*) replacement of the normal bronchial lining cells by metaplastic pavement epithelium may occur.

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giant cells, somewhat simulating the yeasts of *Blastomyces dermatitidis*

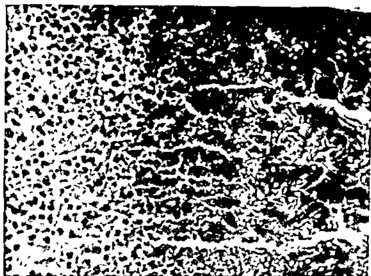


FIG 92 —Bronchial aspergilloma, network of filaments in contact with cellular reaction (Haematoxylin and eosin, ×350)

SUMMARY

Pulmonary aspergillosis occurs in different clinical forms In aspergillary bronchitis the fungous growth is only on the surface of the bronchi In broncho-pulmonary aspergilloma the parasite forms a compact body in a cavity lined by epithelium, the cavity increasing with the growth of the fungus Finally, there is diffuse pulmonary aspergillosis

In cases of aspergilloma in which culture of the fungus was achieved, *Aspergillus fumigatus* was the only organism isolated The morphological appearance of the fungus differed according to its localization in the lung

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2 [illegible] " *J franc*

3 [illegible] megamice-

inhalation, though sometimes the symptoms recurred later. In some cases there was no immediate response, but asthma developed some hours later. In one patient transient diffuse lung shadows appeared after the inhalation (*b*) On occasion systemic reactions occurred, consisting of flushing, pallor, nausea and faintness These were rapidly relieved by isoprenaline inhalation Careful attention to graded concentrations of antigen for inhalation can minimize such reactions

The patients in whose sputum *A. fumigatus* was found fell into the following groups, according to the clinical and pathological picture and to the skin and bronchial reactions on testing

(1) Asthma with allergic sensitivity to aspergillus antigen: (*a*) Recent origin; (*b*) long standing

(2) Aspergillus mycetoma (*a*) With asthma and allergic sensitivity to aspergillus antigen; (*b*) without asthma or allergic sensitivity.

(3) Patients with *A. fumigatus* in the sputum who did not show evidence of skin or bronchial sensitivity

ASTHMA WITH ALLERGIC SENSITIVITY TO ASPERGILLUS ANTIGEN

Included in the clinical features in this group were recurrent transient lung shadows, eosinophilia in the peripheral blood, and the expectoration of plugs containing eosinophile cells and, on occasion, mycelial fragments

Asthma of recent origin.—There were 4 patients in this group. In 2 of these there was a history of wheezing of only 12 weeks' duration. In all cases the bronchi reacted to the aspergillus extract inhalation Prick tests with the aspergillus extract gave a positive reaction in all cases although in 1 the reaction was very weak

Asthma of long standing.—In 8 patients the asthma had been present for many years and had become more frequent and chronic in recent years In all these patients the bronchi reacted to the inhalation of the aspergillus antigen, and positive skin-test reactions were also elicited

A brief outline of the clinical picture is given for some of these patients One patient, 34 years old, had been asthmatic from childhood During the past 6 years transient lung shadows were seen and a left spontaneous pneumothorax had occurred Bronchograms showed marked bronchial dilatation at the sites of previous transient shadows The sputum, which was scanty and mucopurulent, contained mostly eosinophile cells, and no bacterial pathogens were found Skin tests showed a wide spectrum of skin hypersensitivity, positive reactions being elicited by extracts of *Aspergillus*,

DIRECT BRONCHIAL SENSITIVITY TESTS IN BRONCHO-PULMONARY ASPERGILLOSIS

K. CITRON AND J. PEPYS

THIS report describes the investigation, by direct bronchial sensitivity testing, of the possible significance of the presence of *Aspergillus fumigatus* in the sputum. The reactions elicited by the inhalation of antigen extracts were compared with skin reactions to prick tests with these antigens The results obtained suggest that hypersensitivity is of importance in determining the clinical pattern of aspergillus infection of the lung

The technique of the direct bronchial test consists in the administration of inhalations of solutions of various antigens to the patient, and in measuring any resulting bronchial reaction by observing changes in the forced expiratory volume in the first second (F.E V $_{1 \cdot 0}$) This is a reliable measurement of the presence of bronchial obstruction. To start with, recordings of the forced expiratory volume are made on a light spirometer until consistent readings are obtained This is followed by inhalations of a mist of the control material and of the test antigens and the taking of records after each of these

In this study a Bright-Smith nebulizer capable of giving a fine mist (particle size diameter up to 8μ) was employed for the test inhalations which were given for 60 seconds or less An inhalation of Coca's fluid, the vehicle used for extracting the antigens, was given as the control and tracings were made This was followed by inhalations of the antigen extracts Concentrations of 1 and 10 per cent of the antigens were employed and the choice was carefully graded according to the patient's clinical and skin sensitivity Tracings were repeated at intervals up to 15 minutes after the inhalation When a fall in the F E V $_{1 \cdot 0}$ occurred, an inhalation of isoprenaline (1 per cent) was given, and further tracings were made during the recovery period A fall in the mean F E V $_{1 \cdot 0}$ after the inhalation of the antigen, greater than that, if any, following the control inhalation, for the individual patient, was regarded as a positive response

Reactions of the following type were observed after the inhalations (*a*) A fall in the F E V $_{1 \cdot 0}$ with or without symptoms of asthma The asthmatic symptoms were rapidly relieved by the isoprenaline

sensitization to the fungi, though they were unable to provide supporting evidence from skin tests for specific allergic sensitivity. The positive direct bronchial sensitivity test reactions and the skin-test reactions to aspergillus antigen in our cases supports, and adds evidence to, the role of allergic sensitivity to aspergillus antigen in the production of some of the clinical and pathological manifestations. This is particularly pertinent in relation to the bronchial tests

Positive skin-test reactions and clinical allergic reactions to mould spores, including those of the *Aspergillus* species, are widely recognized and commonly encountered In subjects already sensitive or capable of being sensitized, the proliferation of the fungus in the bronchial tract may be expected to excite vigorous tissue reactions Mucosal oedema, bronchospasm and hypersecretion of mucus may contribute to the bronchial obstruction which results in the areas of collapse consolidation so often seen Such areas of collapse may in turn be the cause of the bronchiectasis at the sites of the transient shadows

From the results of this investigation, it is concluded that hypersensitivity to aspergillus antigen plays an important part in determining clinical manifestations

ACKNOWLEDGEMENTS

The cultural studies in this work were performed by Miss Y M Clayton, B Sc, and the antigens employed were prepared by Messrs Duncan-Flockhart & Co Ltd

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Cladosporium, Alternaria, Penicillium, pollen and house dust The inhalation test, however, showed bronchial sensitivity only to *Aspergillus, Cladosporium* and *Alternaria* extracts and not to the other antigens. The most marked reaction was elicited by the aspergillus antigen, with lesser reactions to *Cladosporium* and *Alternaria* antigens. *A. fumigatus* was found in 1 out of 12 sputum cultures

The treatment in this case consisted of inhalations of a brilliant green solution (1 in 10,000), and specific hyposensitization by injection of an aspergillus extract under cover of cortisone. A progressive decline in the bronchial sensitivity to the aspergillus antigen has been demonstrated by serial bronchial sensitivity tests.

In 2 other cases, both long-standing asthmatics, recently incapacitated by the asthma in spite of cortisone treatment in one of them, bronchial and skin-test reactions were elicited by the aspergillus antigen In 1 case *A. fumigatus* was found in the sputum after 17 specimens had been examined, and in the other after 23 specimens. It would seem that intensive search for this fungus is indicated by the sensitivity test reactions

ASPERGILLUS MYCETOMA

With asthma and allergic sensitivity to the aspergillus antigen.— There were 2 patients in this group, showing both bronchial and skin sensitivity to aspergillus antigen In 1 case a mycetoma had developed from a lung abscess, but there were present, in addition, asthma, transient lung shadows and eosinophilia.

Without asthma.—The 2 cases in the group did not react to bronchial tests with aspergillus antigen in contrast to the group with asthma One of the patients had sarcoidosis and a mycetoma, but no asthma or eosinophilia The bronchial test was negative in this patient although a positive skin-test reaction was elicited

PATIENTS SHOWING NO EVIDENCE OF SKIN OR BRONCHIAL SENSITIVITY

In 7 cases, A fumigatus was found in the sputum, but it was not possible to relate its presence to the clinical condition since both skin and bronchial tests were negative

The patients with asthma of recent onset and with mycetoma referred to above are similar to those described by Hinson, Moon and Plummer[1] under the term broncho-pulmonary aspergillosis They divided their cases into a saprophytic, an allergic and a septicaemic group The saprophytic and allergic types comprise the patients described here They emphasized the importance of

dust, he gradually improves both clinically and radiologically and he should be able to return to work in 2–4 months

Repeated episodes of this kind described in the second stage of disease gradually give rise to permanent scarring of the lung with fibrosis and occasionally bronchiectasis. Unless it has been possible to watch a case over a period of years a firm diagnosis in this third stage cannot be made, for it is impossible to differentiate it from many other causes of pulmonary fibrosis

AETIOLOGY

The possibility that farmer's lung was a simple allergy was considered[4] and solutions from hay samples and moulds were made by extracting 1 gramme of chopped hay in 25 millilitres of sterile water overnight at 2° C. and then Seitz-filtering the extract Similar extracts were made from 2-week to 4-week fluid cultures of individual moulds in glucose medium Extracts of hay, whether good or mouldy, gave fairly constant positive results with intradermal tests in both farm workers and in a control subject, the response to mould extracts was poor. It seemed likely that the hay extracts contained a substance giving a histamine-like response Evidence for simple allergy being responsible was unconvincing

The sera of a significantly large number of these patients showed an increase in the *gamma*-globulin fractions This could be interpreted as the result of the production of antibodies of unspecified nature, but was not regarded as of any diagnostic value Mantoux tests were done in 12 cases and only 2 (16 per cent) were positive, as against 63 9 per cent of positives in the rural adult population in Devonshire

The significance of moulds in the sputum of these patients is very doubtful, and histological studies of the lung seemed of paramount importance, a paper by Zettergren in 1950 appeared to be of significance in this respect[6] The publication described the exposure of one group of rabbits to an atmosphere containing heavy concentrations of sterile threshing dust and another to sterile threshing dust containing *C albicans* Autopsy on the first group of rabbits showed "dust granulomas" in the lungs with foreign-body giant cells, in the second group a tuberculoid reaction ("fungal granulomas") occurred

Histological studies were carried out on 2 patients on whom lung biopsy was performed

Case 1 —A young agricultural worker aged 27 years had been using mouldy hay to feed his cattle for one month prior to hospital admission

FARMER'S LUNG

C J. FULLER

FARMER'S or thresher's lung was first described in England by Campbell in 1931[1] Fawcitt, working in Cumberland and Westmorland, described further cases and carried out investigations from which he deduced that the condition was a bronchomycosis[2,3]. Tornell in Sweden published a further paper in 1945, in which he came to the conclusion that *Candida albicans* was the responsible fungus[5].

Observations upon this condition seen in Devonshire were published in 1953[4]. It was shown that the presence of fungi in sputa from these patients did not prove the condition to be a true bronchomycosis, particularly as farm workers lived in an atmosphere laden with mould spores Bronchoscopy of active cases showed some slight reddening of the bronchial mucosa, and bronchial swabs gave negative cultures in 3 and a growth of *Penicillum* sp in one. It was suggested that farmer's lung was due to a tissue reaction to breakdown products of inhaled mould spores or grass particles

CLINICAL FEATURES

Farmer's lung can occur as an acute illness following exposure to heavy concentrations of hay or threshing dust The symptoms are manifest in a few hours, as fever, malaise, shivering and a dry cough A small amount of sticky sputum, sometimes blood-stained, may be [illegible]

The chief symptom then is breathlessness severe enough to prevent work, and cough productive of a little purulent sputum The patient is cyanosed, and fine crepitations are audible over the lung bases and breath sounds are weak Radiological findings are characteristic and show fine mottling, particularly in the middle and lower lung zones, the apices usually show some degree of emphysema.

Provided the farm worker is kept away from further contact with

Differential diagnoses considered in Case 1 included tuberculosis and sarcoidosis and in Case 2 dust pneumonitis In neither case was there any helpful evidence of mould spores or fungi growing in the lung tissue.

There is a similarity between Zettergren's "fungal granulomas" and the tuberculoid systems in Case 1, and between his "dust granulomas" and the foreign-body giant cell systems in Case 2. It is suggested that in farmer's lung a peculiar cellular reaction occurs in the lung tissue as a result of the inhalation of dust and mould spores giving rise to the clinical and radiological changes recorded in this disease.

ACKNOWLEDGEMENT

Acknowledgement is made to Dr G Stewart Smith for his histological reports quoted above

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He was admitted to Exmouth Hospital on April 11th, 1956, with a history of breathlessness and cough. His Mantoux was negative at 1:1000, and in view of extensive mottling throughout both lungs a tentative diagnosis of farmer's lung was made. Lung biopsy was carried out on May 3rd, 1956, and at operation the surface and substance of the lung showed miliary bodies indistinguishable from miliary tuberculosis. Half the biopsy specimen was examined bacteriologically and half histologically. *Mycobacterium tuberculosis* was not isolated in culture nor by animal inoculation, nor was it found in repeated examinations of sputum, bronchial washings and gastric residues during the 3 months following operation. Histological examination of the biopsy material showed a large number of tuberculoid systems. These contained endothelial cells, and sometimes giant cells, in the periphery with a central ring of small round cells. Some of these systems showed central necrosis, but many of them did not contain giant cells, and simulated a sarcoid reaction. No foreign bodies were seen in the giant cells, nor was any doubly refractile material or dust present. Lipophages were numerous.

Clinical and radiological recovery followed and the patient returned to work 3 months after the biopsy was taken, he has been well since and a radiograph taken 10 months later was normal.

Case 2.—A man aged 55 years had helped to thresh a rick of mouldy corn in a barn during January 1954. The same evening he developed a "chill" as did 2 other members of the threshing team to a lesser degree. A month later the patient was examined and was found to be suffering from exertional dyspnoea and fatigue. Crepitations and rhonchi were apparent and a radiograph showed a broad mediastinal shadow and areas of fine mottling at the lung bases. After bronchoscopy and tomography, surgical exploration of the right chest was carried out and a biopsy of lung taken, a retrosternal and partially calcified thyroid was observed. Histology of the biopsy material showed only an occasional focus of endothelial cells with a few giant cells. The tissue was, however, infiltrated with masses of small round cells including small numbers of plasma cells. Many scattered giant cells were present, mostly of foreign-body type containing spear-shaped clefts resulting from dissolved foreign-body material, possibly cholesterol. A small amount of brownish dust material was present, not within giant cells, but there were no doubly refractile particles. Lipophages were plentiful. The condition was considered to be a type of chronic pneumonitis around bronchioles which could well be a reaction to dust.

The patient made very slow progress towards resuming his life as an active farmer. Three months after the thoracotomy he was unable to

reviewed by Parsons and Zarafonetis in 1945 were all that had been observed[34]. In contrast, in recent years the Public Health Service, Armed Forces, Veterans Administration and a number of universities have vigorously pursued investigations into the prevalence, pathology, distribution and therapy of this disease. Moreover, the geographic distribution of *H. capsulatum* is not well defined, either in the United States of America or in the many other countries where surveys for histoplasmin sensitivity have been carried out[14]. Great Britain, of course, is among those areas in which the degree of endemicity is not k--

in

repeatedly observed in certain types of the infection will be described, and the way in which serology may be used in detecting primary infections and in mapping geographic areas of high endemicity will be illustrated.

SEROLOGICAL TESTS

The complement fixation test and the collodion agglutination test are the two serological procedures employed for histoplasmosis at the Walter Reed Army Institute of Research The methods are described in detail in earlier reports[7, 39].

In the complement fixation (CF) test two antigens are used, a standardized suspension of the whole yeast phase of *H capsulatum* in buffered saline solution, and histoplasmin[23] The latter is a filtered asparagine broth medium in which selected strains of *H capsulatum* in the filamentous phase are grown for several months The same type of preparation, following appropriate standardization, is used for skin testing and the collodion agglutination procedure

ADVANTAGES OF THE COLLODION AGGLUTINATION TEST

The collodion agglutination (CA) test employs as antigen minute particles coated with the non-particulate antigen, histoplasmin (Fig 93) The particles require 1 hour for sensitization after which they are agglutinated in the presence of serum from early cases of histoplasmosis Serial dilutions of sera to be tested are made and to these antigen is added After incubation for 2 hours, the tubes are centrifuged and read by the conventional 4 plus, 3 plus method One person can routinely complete 100 tests in their entirety within a period of 4 hours Some laboratories use the more time-consuming and less economical precipitin test instead[38].

SEROLOGICAL DIAGNOSIS AND EPIDEMIOLOGICAL ASPECTS OF HISTOPLASMOSIS

CHARLOTTE C. CAMPBELL

WITHIN the past decade there has been a revolutionary change in the clinical concept of histoplasmosis. Once regarded as a rare, slowly progressive and invariably fatal infection, it is now only the fatal outcome that is considered unusual among the countless persons who experience primary pulmonary infection.

Infection follows inhalation of spores of *Histoplasma capsulatum*, a fungus which exists saprophytically in the soils of many defined, and some as yet undefined, geographic areas. The first evidence of the benign infection came from the use of histoplasmin, an antigen derived from the causative fungus, in skin test surveys of healthy residents of the central United States of America who, despite pulmonary calcifications, failed to react to tuberculin[9, 30]. The nature of the benign illness, or other mechanism leading to the widespread production of this sensitivity, was not known at that time In a few years, however, there was conclusive evidence that primary histoplasmosis was an acute pneumonitis clinically indistinguishable from other and supposedly more common pneumonitic diseases of viral or bacterial aetiology. As in the latter infections, there was an acute onset with fever, malaise, sometimes nausea and vomiting, dyspnoea and dry cough. Radiological examination of the chest revealed varying degrees of unilateral or bilateral infiltration present in one or more lobes The close study of persons involved in outbreaks or epidemics, and of increasing numbers of individual cases, further disclosed that such infections ranged from the clinically inapparent to the extremely severe

While most persons apparently recovered, there were others in whom unhealed pulmonary lesions persisted and some in whom widely disseminated progressive disease terminated in death In brief, the clinical spectrum of histoplasmosis is as broad and varied as that of many other infectious diseases

There is, as yet, little evidence that histoplasmosis is a problem of importance in Great Britain Nevertheless, it should be remembered that little more than 10 years ago this was also being said of the United States of America. The 78 fatal or moribund cases

TABLE 31

SEROLOGICAL REACTIONS IN PRIMARY PULMONARY HISTOPLASMOSIS

Days after onset	*Complement fixation titre**	*Collodion agglutination titre (Histoplasmin)*
6	Negative	1:256
12	Negative	1:256
19	Negative	1:256
25	1:32	1:256
32	1:32	1:256
62	1:32	1:128
120	1:16	1:64

* Whole yeast phase antigen   Skin reactions with histoplasmin negative throughout

TABLE 32

TYPICAL SEROLOGICAL REACTIONS IN SERIAL SPECIMENS FROM PATIENTS WITH PRIMARY PULMONARY HISTOPLASMOSIS

Case No	*Date after onset*	*Complement fixation titre*		*Collodion agglutination titre (Histoplasmin)*
		*WYP**	*H†*	
3		1:256	1:128	1:64+
	2 weeks later	AC‡	AC	1:32
	4 weeks later	1:256	1:128	1:16
6		AC	AC	1:32
	3 weeks later	Negative	1:64	1:8
11		Negative	Negative	1:64
	4 weeks later	Negative	1:64	Negative

* WYP Whole yeast phase antigen of *H. capsulatum*
† H Histoplasmin
‡ AC Anticomplementary specimen

acquired by the casual passer-by as well as those who work directly with the organism

In Table 32, additional advantages of the test are illustrated. The first serum samples in cases 6 and 11 were anticomplementary and CF negative respectively, but because of the strong collodion reactions additional studies were made. Subsequent specimens on both of these patients had demonstrable CF antibody titres of 1:64 to histoplasmin

DIFFERENCES IN THE REACTIVITY OF THE TWO ANTIGENS USED IN THE COMPLEMENT FIXATION TEST

Complement fixing (CF) antibodies, although sometimes developing much later than agglutinins or precipitins, usually persist for

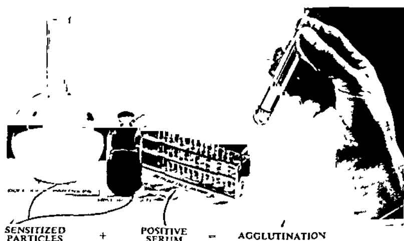


FIG 93—The collodion agglutination test (*By courtesy of Medical Audio Visual Dept., Walter Reed Army Institute of Research, Washington*)

The collodion test has many advantages over other serological tests. It may be performed with ease and rapidity It may be used to demonstrate the presence of antibody in cases as early as 10–14 days after exposure to the fungus, usually by the time clinical symptoms are manifest. Moreover, agglutinins are observed in mild cases in which CF antibodies are never demonstrable, or do not arise until much later Finally, the test is of value in sera which are anticomplementary Agglutinins, like precipitins, are transitory and are seldom demonstrable in the chronic, slowly progressive cases The collodion test, therefore, is always used in conjunction with, and not in the place of, the CF test

These advantages are illustrated in Tables 31 and 32 The patient represented in Table 31 was an entomologist, aged 32 years, whose influenza-like episode was of 5 days' duration The serum was tested on the sixth day after he returned to work and gave a strongly positive collodion agglutination test, but CF antibodies were not demonstrated until 2 weeks later when the patient had recovered clinically and after culture studies of the sputum, from which *H capsulatum* was isolated, were initiated Histoplasmosis was suspected in this case because of the patient's frequent visits to our laboratory The case is thus illustrative not only of the advantage of the CA test in detecting the primary case, but of the highly infectious nature of the agent. Laboratory infections can be

of 1:32–1:512 with the whole yeast phase antigen that failed to react with histoplasmin. There was a third group of comparable size in which the reactions were essentially the same with both preparations[23]. In other words, it is estimated that sera from approximately one-third of the histoplasmosis cases would yield false negative CF reactions if one preparation were used to the exclusion of the other Although the whole yeast antigen appears to be more highly reactive in sera from primary cases, and histoplasmin more so in sera from persons with residual lesions, cavitation or fibrosis, notable exceptions are observed even to these broad generalizations

A much greater number of cases must be followed serologically from onset before an attempt can be made to interpret these differences, and for the present both types of preparation in the CF test should be used

THE USE OF SEROLOGICAL TESTS IN EPIDEMIOLOGICAL STUDIES

While serological tests are perhaps most useful in detecting primary infections, they frequently are also the means of indicating possible new areas of high endemicity Histoplasmosis in what are presumed to be non-endemic areas is usually first recognized by visiting physicians, or those new to the community, who request serological tests that lead to its early diagnosis When it is further ascertained that the patient has neither lived nor travelled in areas known to be endemic, a source of infection is looked for in his immediate environment This, if found, leads to more definitive studies to determine the real prevalence of the infection in a geographic area in which the degree of endemicity is not known

Such studies remind us that histoplasmosis is not a disease limited to the central United States of America, an impression that, unfortunately, has arisen because of the impressive investigations on histoplasmosis in that region It is sometimes quite difficult to persuade physicians with practices outside the central United States of America that their patients have this "middle western" disease[31]

The following case, in addition to the serological findings observed in many primary infections, exemplifies this sequence of events

The patient, a white male research chemist aged 26 years, had always been in good health One evening he developed a "chill" and sore throat and the following day his temperature was 103° F. and he suffered from rigors Attacks of sweating, shortness of breath on the slightest exertion and a dry cough followed He was treated with a variety of antibiotics and when there was no improvement after two weeks he

much longer periods. Particularly in non-primary cases, therefore, only the CF test is positive Moreover, there are sometimes wide and as yet inexplicable differences in the reactivity of the whole yeast phase and histoplasmin antigens in the CF test. It is for this reason that both are used in this procedure.

The extent to which reactions with the two antigens vary is exemplified in Tables 33 and 34. Recently, complement fixation reactions were compared on the thousand sera most recently sent to our laboratory to be tested routinely for blastomycosis, coccidioidomycosis and histoplasmosis[23]. Of these, there were 146 specimens that fixed complement to titres of 1:32 or above with histoplasma antigens It will be noted, however, that of the 41 sera listed in Table 33, 30 were negative with the whole yeast antigen but reacted to CF levels of 1:32–1:256 with histoplasmin. Of the 57 sera in Table 34, on the other hand, there were 34 that reacted to CF levels

TABLE 33

COMPARISON OF COMPLEMENT FIXATION TITRES OF 41 SERA (33 CASES) IN WHICH HISTOPLASMIN REACTED TO HIGHER TITRE THAN WHOLE YEAST ANTIGEN (WYP)

Number of sera	*CF titre with WYP antigen*	*Number of sera with CF titre to histoplasmin at*			
		1:32	*1:64*	*1:128*	*1:256*
30	1:8	14	10	4	2
5	1:8	4		1	
5	1:16		5		
1	1:32				1

TABLE 34

COMPARISON OF COMPLEMENT FIXATION TITRES IN 57 SERA (32 CASES) IN WHICH WHOLE YEAST PHASE ANTIGEN (WYP) REACTED TO HIGHER TITRE THAN HISTOPLASMIN

Number of sera	*CF titre with histoplasmin*	*Number of sera with CF titre to WYP antigen at*				
		1:32	1:64	1:128	1:256	1:512
34	1:4	19	8	4	1	2
5	1:8	2		1	2	
11	1:16		3	3	3	2
6	1:32			5	1	
1	1:64					1

of 1:32–1:512 with the whole yeast phase antigen that failed to react with histoplasmin There was a third group of comparable size in which the reactions were essentially the same with both preparations[23]. In other words, it is estimated that sera from approximately one-third of the histoplasmosis cases would yield false negative CF reactions if one preparation were used to the exclusion of the other. Although the whole yeast antigen appears to be more highly reactive in sera from primary cases, and histoplasmin more so in sera from persons with residual lesions, cavitation or fibrosis, notable exceptions are observed even to these broad generalizations.

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The following case, in addition to the serological findings observed in many primary infections, exemplifies this sequence of events

[illegible]

antibiotics and when there was no improvement after two weeks he

was admitted to hospital Radiological examination of the chest revealed "dense, diffuse miliary disease which was grossly nodular" (Fig 94) and anti-tuberculosis therapy was initiated. On the following day a visiting physician familiar with histoplasmosis saw the patient and serological tests were advised.

As indicated in Table 35, both the CA and CF tests were strongly positive. Culture studies were immediately initiated and *H capsulatum* was recovered from gastric washings and sputum collected on the third day of hospitalization The patient improved rapidly and was discharged after 1 month. Radiographs of the chest 3 years later revealed many tiny calcified nodules, now regarded as characteristic of healed miliary histoplasmosis The patient maintains a residual CF titre of 1.8 with the whole yeast phase antigen and a strongly positive skin test



Fig 94 —Pulmonary histoplasmosis Radiograph showing diffuse nodular densities throughout both lung fields (*By courtesy of Dr James S Feffer, Washington*)

It should be noted that the patient's CF titres were not only higher but persisted for a much longer period with the whole yeast phase [illegible] histoplasmin. Agglutinins were no longer demon-

Because the same is true of CF antibodies in some of the milder

cases, sera should be tested early and often in suspected cases The marked cross reaction with blastomyces antigen is discussed in a later section

TABLE 35

SEROLOGICAL REACTIONS IN PRIMARY PULMONARY HISTOPLASMOSIS *H. CAPSULATUM* ISOLATED FROM PATIENT AND SOIL TO WHICH HE WAS EXPOSED

Period after onset	*Complement fixation titre*				*Collodion agglutination titre (Histoplasmin)*
	*WYP**	*H†*	*B‡*	*C§*	
16 days	1 512	1·64	1.512	Negative	1.32
23 ,,	1 256	1 16	1 256	Negative	1 32
30 ,,	1 256	1 32	1 128	Negative	1 32
39 ,,	1·256	1 8	1 128	Negative	1.16
52 ,,	1 256	1 16	1:64	Negative	1:8
67 ,,	1 128	Negative	1 32	Negative	Negative
3 years	1 8	Negative	Negative	Negative	Negative

* WYP — Whole yeast phase antigen of *H capsulatum*
† H — Histoplasmin
‡ B — Yeast phase antigen of *Blastomyces dermatitidis*
§ C — Coccidioidin

There is little doubt, however, that it was the serological tests which provided the rapid, presumptive diagnosis during the active phase of this infection when cultural studies, which are so rarely carried out for *H capsulatum*, are also most rewarding Finally, when it was assured that the patient had neither served in the Armed Forces nor resided nor travelled beyond the north-eastern section of the United States of America, the source of his exposure was sought and found in his own garden Ten days before onset of illness, he had cleared an area on which an old chicken house had stood The soil samples collected from this area were laden with spores of *H capsulatum*[4] Moreover, a skin test survey of the county in which the patient resided revealed a high dermal sensitivity rate to histoplasmin[2], indicating that other cases of primary infection were being overlooked or attributed to other causes in this area

EPIDEMIC HISTOPLASMOSIS

Histoplasmosis occurs in epidemics when a number of persons are exposed to a common source in Nature Furcolow and his associates have studied approximately 30 of these outbreaks[19, 40] in addition to those described by Looslı and others[3, 8, 22, 28, 29, 33] Epidemics usually come to be recognized only when at least one of the patients is sufficiently ill to require admission to hospital For this reason, it is believed that even in areas known to be endemic many of the less severe cases are not detected

The association between awareness and known prevalence of histoplasmosis is well-known. The epidemics reported from New York[22], Maryland[3, 28], North Carolina[33], or even Venezuela[8] differ from those described by Loosli and Furcolow[19, 29] only in that they occurred in areas thought to be of low endemicity, but where few or no skin test studies have actually been done to determine the real prevalence of the infection.

In these outbreaks the serological test was used to good advantage. For example, an otherwise healthy butcher, aged 32 years, was admitted to a hospital in the District of Columbia with a severe, acute pneumonitic illness similar to that described in the preceding case (Figs. 93 and 94, and Table 35) A probable diagnosis of psittacosis was made, but positive serological reactions were observed with histoplasma antigens and not with those of the appropriate group of viruses Moreover, it was learned that three other members of this man's family were experiencing milder but similar episodes of illness His son recovered following 2 weeks of bed-rest at home, his wife after only 1 week, and another relative missed only several days at work because of "a very bad cold". As shown in Table 36, agglutinins were demonstrated in the sera from all 3 persons with the milder infections, as well as the father's whose grave illness required 3 months of hospital treatment Complement

TABLE 36
SEROLOGICAL REACTIONS IN A FAMILY OUTBREAK OF HISTOPLASMOSIS[17]

Case	*Days after onset*	*Complement fixation titre*		*Collodion agglutination titre (Histoplasmin)*
		*WYP**	*H†*	
1 (Father)	15	1 64	Negative	1.64+
	41	1 64	Negative	1 16
	197	1 8	Negative	1 16
2 (Mother)	10	Negative	Negative	1 64
	43	Negative	Negative	1 64
	191	Negative	Negative	1 8
3 (Son)	10	1 64	Negative	1 32
	24	1 256	1 32	1 64
	191	1 128	1 256+	1 8
4 (Sister-in-law)	13	1.8	Not done	1 8
	27	1 64	Not done	1 64
	194	Negative	Not done	Negative

* † See footnote to Table 35

fixing antibodies were demonstrated with the whole yeast phase antigen in Case 1, and also to high titres of 1:128 and more than 1:256 with both antigenic preparations in Case 3, but were not demonstrable with either in Case 2. Prompted by the positive agglutination test, cultural studies were carried out and *H. capsulatum* was isolated from the sputum of Case 2 as well as of Case 1[28].

The organism was also later isolated from the soil to which all 4 of these persons were exposed 10–14 days before the onset of illness. The soil had been contaminated with chicken excreta used as fertilizer[3]. This outbreak occurred within 5 miles of the city limits of the District of Columbia, where endemicity at that time was regarded as low. Since the time of this outbreak 64 cases of primary pulmonary histoplasmosis in local residents have been found with the help of serological tests, and in a recent unrelated skin test survey in one of the adjoining counties a high dermal sensitivity rate to histoplasmin was revealed[13]. The positive serological tests, and the isolation of *H. capsulatum* from local soils associated with a number of these cases[5, 16] had, in fact, long suggested that histoplasmosis was far more prevalent in these areas than was indicated by the few and very limited skin test studies previously made in this region.

SEROLOGICAL TESTS IN AREAS OF HIGH ENDEMICITY

Serological studies are also helpful in regions where, for various reasons, histoplasmosis is believed to be endemic, even though the primary infections are not recorded. The Isthmus of Panama, where the first 3 cases of histoplasmosis, all fatal, were described by Darling in 1906, is a case in point[10]. Although a fourth case was not reported from Panama until 1951[12] there have since been several others, all of the fatal type[35]. In addition, Tucker[42] reported a dermal sensitivity rate of more than 60 per cent in the indigenous population, and Ajello isolated *H. capsulatum* from Panamanian soil[1]. Despite these findings there were no reports of the primary infection.

In 1955 the Walter Reed Army Institute of Research began a co-operative study with physicians at Gorgas Hospital on the isthmus. Within a period of 1 year positive serological reactions were obtained in 81 patients with acute pneumonitic infections who were admitted to the chest diseases wards at that institution. As shown in Table 37, there were 60 cases on whom only a single serum was tested and a total of 15 sera that were anticomplementary. Nevertheless, the latter were all positive in the CA test, as were a number of others with low CF titres of 1:8–1:32. Complement

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	41	1 64	Negative	1 16
	197	1 8	Negative	1 16
2 (Mother)	10	Negative	Negative	1 64
	43	Negative	Negative	1 64
	191	Negative	Negative	1 8
3 (Son)	10	1 64	Negative	1 32
	24	1 256	1 32	1 64
	191	1 128	1 256+	1 8
4 (Sister-in-law)	13	1 8	Not done	1 8
	27	1 64	Not done	1 64
	194	Negative	Not done	Negative

* † See footnote to Table 35

SEROLOGICAL TESTS

The conversion of skin tests from negative to positive is another primary criterion for determining whether a recent infection is one of histoplasmosis. However, as exemplified by the cases in Table 38, conversion does not always occur during the earliest stages of the infection Humoral antibodies frequently are demonstrable much earlier than skin test conversion.

SEROLOGICAL TESTS IN POST-PRIMARY HISTOPLASMOSIS

In order to counteract the early concept that histoplasmosis was rare and invariably fatal, its benignity has perhaps been overstressed, leaving many clinicians with the impression that the morbidity it produces is negligible While this is true in many cases, there is increasing evidence that problems relating to the treatment of residual and often undetected infections with *H capsulatum* now surpass those in tuberculosis The development of special histological stains for fungi[20, 21, 27] led to the discovery that many of the granulomatous lesions heretofore attributed to tuberculosis were, in fact, caused by *H capsulatum*[11, 37]. "Histoplasmomas" are being found in an increasing number of asymptomatic and mildly symptomatic individuals whose multiple or single unhealed lesions are first discovered in routine radiological examinations In many others, there is cavitation and fibrosis Finally, there are the frankly disseminated and probably incurable infections. To these must be added persons with histoplasmosis associated with sarcoidosis[24, 25, 36] and, as recently pointed out by Furcolow in his study of patients in sanatoria, those with tuberculosis and histoplasmosis[18]

The serological patterns observed in non-primary cases vary to some extent with the type, duration and sometimes severity of disease, but it is still impossible to relate serological findings with prognosis[6] The sera of some chronic cases with pulmonary or extra-pulmonary lesions, and even of some persons with disseminated disease, give negative serological results On the other hand, there are others with complement fixing titres exceeding 1 2,048 at the time of death[5]. While a negative reaction is not a criterion for excluding infection, a positive one often provides the first clue that a chronic and undetermined illness is one of histoplasmosis

The reactions recorded in Table 39 were observed in a chronically ill person, and indicate that serological tests and the testing of serial specimens are frequently contributory to the diagnosis of the post-primary case

This patient was a well-developed though chronically ill Negro female, aged 35 years, who was admitted to the hospital with abdominal

TABLE 37

SEROLOGICAL RESULTS IN 108 SERA FROM 81 PERSONS WITH PRESUMPTIVE PRIMARY PULMONARY HISTOPLASMOSIS (PANAMA CANAL ZONE)

Number of cases	*Specimens*	*Number of sera*	*Complement fixation test*		
			Positive	*Negative*	*Anticomplementary*
60	Single sera				
	1. Collodion positive	35	16	14	5
	2 Collodion negative	20	20	0	0
	3 Collodion not done	5	5	0	0
21	Multiple sera (48)				
	1 Collodion positive	32	15	7	10
	2 Collodion negative	16	16	0	0

TABLE 38

SKIN TEST F ... LY PRESUMPTI\ ... ES AND GOVER ... HE PANAMA

Number of cases	*Skin tests with PPD*	*Histoplasmin skin tests*		
		Positive first test	*Converted (4–14 weeks)*	*Negative*
28	Negative	18	9	1
17	Positive	12	5	0
45		30	14	1*

* Disseminated case

fixing titres in the majority, however, ranged from 1·64 to 1·256 Unfortunately, cultural studies were not done, but as shown in Table 38 additional data in 45 of these cases reported by Cleve and Young revealed that skin test reactions to histoplasmin converted from negative to positive in 14 of the 45 persons, or 31 per cent, during a 4–14 week period of observation[43] *H capsulatum* was also later isolated from one of the patients with a CF titre of 1.32 when he was returned to the United States of America because of a residual lung lesion[43] On the basis of positive serological reactions it is now estimated that, of the hundreds of cases admitted to Gorgas Hospital with what, prior to this study, was termed "pyrexia of unknown origin", primary pulmonary histoplasmosis comprised a high proportion.

ADDITIONAL EPIDEMIOLOGICAL CONSIDERATIONS

The distribution of *H. capsulatum*, even in areas known to be endemic, appears to be patchy rather than continuous[45] It is, therefore, possible to find a high dermal sensitivity rate in one section of a small county and a low one only a few miles distant. This may be attributed to certain reservoirs that meet the nutritional requirements of the fungus in the one area but not in another. These requirements are provided in soils high in nitrogenous content, plentifully supplied with moisture and protected from the direct rays of the sun[15], such as may be found in caves, wooded areas, cellars, clock towers, silos and old chicken houses[3, 8, 19, 22, 28, 29, 33] The latter have been associated with an impressive number of cases of histoplasmosis since the affinity of *H capsulatum* for soils contaminated with the excreta of chickens or other fowls was first pointed out by Zeidberg and Ajello in 1952[44].

Histoplasmosis is, however, not entirely a disease of rural inhabitants Five of the 7 cases briefly presented in this report were residents of suburban housing developments which were farmlands only a few years before In these new communities throughout the United States of America, gardening is a common pursuit for which chicken compost is in great demand Primary histoplasmosis is therefore kept in mind in suburban and urban residents[26] as well as in rural communities

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symptoms of 3 months' duration Her temperature varied from 102° to 105° F., and there was generalized infiltration throughout both lung f

fungous antigens, and when blastomycin gave a positive result serological tests were carried out In 2 specimens taken over a 5-day interval, the agglutination test was positive for histoplasmosis, while the CF reactions were inconclusive with both antigens for this disease and positive with the blastomyces antigen at a level of 1:64 (Table 39) Six weeks later a third serum sample was sent and on this occasion a significant CF titre of 1·256 was obtained with histoplasmin, while that with the blastomyces antigen had decreased to 1:16 Belated culture studies led to the isolation of *H capsulatum* from the patient's gastric washings and sputum on three separate occasions. This patient, now ambulatory, does not appear to be clinically worse, although her CF antibody has increased to the very high titre of 1:1,024.

THE PROBLEM OF SEROLOGICAL CROSS-REACTIONS

The fungi that produce the systemic mycoses have common antigens that cross-react in both skin and serological tests[6, 17, 32, 41]. Blastomyces antigen is especially troublesome in this regard, cross-reacting to a marked degree with the sera of some patients with histoplasmosis (Tables 35 and 39) and not at all in others. Cross-reactions, though common and often difficult to interpret, do not invalidate the usefulness of these tests, and even where they are most extensive, attention may be directed to the possibility of a mycotic infection so that cultural and additional serological studies can then be more intensively pursued

TABLE 39

SEROLOGICAL REACTION IN NON-PRIMARY HISTOPLASMOSIS ASSOCIATED WITH SARCOIDOSIS

Date after onset	*Complement fixation titre*				*Collodion agglutination titre (Histoplasmin)*
	*WYP**	*H†*	*B‡*	*C§*	
16 weeks (‖)	Doubtful	Negative	1:64	Negative	1:64
17 ,,	Doubtful	Doubtful	1:64	Negative	1:128
23 ,,	Negative	1:256	1:16	Negative	1:32
25 ,,	Negative	1:512	1:8	Negative	1:32
29 ,,	Negative	1:1024	Negative	Negative	1:16
32 ,,	Negative	1:1024	Negative	Negative	Negative

*, †, ‡, § See footnote to Table 35
‖ Antigen insufficiency, test unsatisfactory

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HISTOPLASMIN TESTING IN DIFFERENT GEOGRAPHIC AREAS

PHYLLIS Q EDWARDS

HISTOPLASMIN testing is a most useful epidemiological tool for defining the geographic distribution and prevalence of infection with *Histoplasma capsulatum*. It is important to recognize, however, that not all histoplasmin sensitivity is caused by histoplasmal infection and to distinguish, where possible, specific from non-specific reactions. For histoplasmin testing, as for tuberculin, a standardized antigen is essential for the interpretation of results and for comparison of findings from one area to another. General application of the traditional millimetre criterion for a positive reaction may give, as in reactions to tuberculin, misleading results in some areas By carefully measuring all reactions and presenting the results as percentage frequency distributions, the pattern of the distribution can be made which provides a basis for making the distinction required between specific and non-specific histoplasmin sensitivity Additional evidence can be obtained from study of the frequency of pulmonary calcifications

The map shown in Fig 95 was prepared one year ago and shows where histoplasmin testing surveys had been carried out and the percentage of reactions reported as positive in each locality[2]. It illustrates that sensitivity to histoplasmin is prevalent in the Americas, particularly in the east-central part of the United States of America, the Central American countries, and some regions of South America Europe seems to be free, but so far as can be judged from the few scattered surveys reported from Africa and Asia, low prevalence endemic areas may exist in east-central Africa, perhaps also in the Union of South Africa, and in some parts of south-east Asia (Indonesia, the Philippines and Viet-Nam) The map was compiled from data in which the percentages of positive reactions were reported in the traditional form as reactions measuring more than a specified size, usually 5 millimetres

SKIN SENSITIVITY

Both animal experiments and studies in man have shown that skin sensitivity to histoplasmin may result from infection with other fungus organisms[4, 5, 9]. We know, for example, that persons

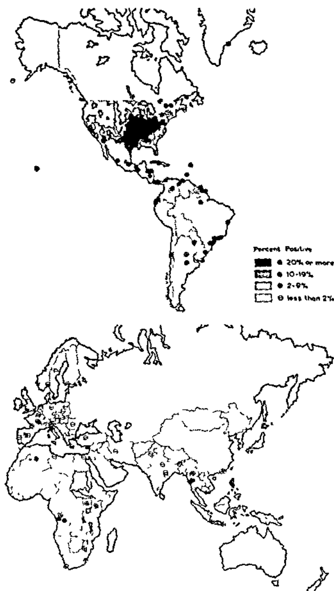


Fig. 95 —Reported prevalences of histoplasmin sensitivity in different parts of the world[2] (*By courtesy of the Editor of Amer J trop Med Hyg*)

infected with *Coccidioides immitis* often react to histoplasmin as well as to coccidioidin Histoplasmin sensitivity due to infection with other fungi is also thought to occur. In order, then, to define the geographic distribution and prevalence of histoplasmal infection, it is necessary to distinguish between the histoplasmin sensitivity caused by histoplasmal infection, which may be referred to as specific sensitivity, and that caused by other fungus infections, or non-specific sensitivity. Results of testing in different geographic areas have revealed striking variations in the pattern of response to histoplasmin[1, 3, 8]. One characteristic pattern of reactions is found in areas where histoplasmosis is prevalent, as evidenced by clinical disease and high frequencies of pulmonary lesions and calcifications in persons who react to histoplasmin but not to tuberculin Another and quite different pattern characterizes the sizes of the reactions of an essentially negative population, where neither histoplasmosis nor coccidioidomycosis nor any other sensitizing agent appears to be present. Still another kind of pattern is found in areas where coccidioidomycosis or some other cross-sensitizing infection is prevalent but histoplasmosis is not.

These several different kinds of patterns are illustrated in material collected by the United States Public Health Service during a two-year period from 1949 to 1951 During that period, young men from all parts of the country were examined radiologically and tested with histoplasmin, coccidioidin and tuberculin as they arrived at a training centre in California for military service. Most of the histoplasmin tests were given by intradermal injection of 0·1 millilitre of a 2 1,000 dilution of the product identified as H-40, prepared by the Public Health Service Coccidioidin C-24, prepared by Dr Charles E Smith, and the international standard PPD tuberculin, PPD-S, prepared by Dr Florence Seibert, were used for the other two tests The testing was done by a team trained to use uniform techniques and to measure and record the size of the induration of the reactions

Results obtained from nearly 40,000 white men between the ages of 17 and 21 years who had lived all their lives in their home counties were used to study the response to histoplasmin in different geographic areas throughout the country[3, 6, 8]

In the United States of America histoplasmin sensitivity is highly prevalent in the east-central area, extending roughly from the Mississippi valley in the west to the Appalachian mountains in the east, from the Great Lakes in the north to the Gulf of Mexico in the south (Fig 96) In some localities within that area, more than 80 per cent of the young adults have histoplasmin reactions measuring

5 millimetres or more. Clinical cases of histoplasmosis are diagnosed frequently in that area and subclinical cases with benign pulmonary infiltrations or calcified lesions are very common. The area is endemic for histoplasmosis Most of the eastern coastline, as well as the entire north-west, is essentially free from this fungous infection, and in the south-west, where coccidioidomycosis is endemic, the prevalence of histoplasmin reactions measuring 5 millimetres or more exceeds 30 per cent in some areas.

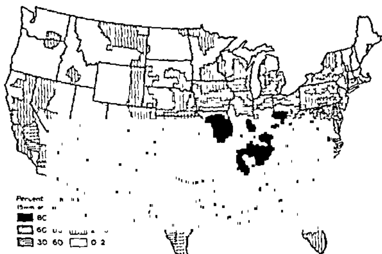


Fig 96 —Prevalence of histoplasmin sensitivity in young adults (*By courtesy of the Editor of Dis Chest*)

Fig 97 shows the percentage of coccidioidin reactions measuring 5 millimetres or more throughout the United States of America The high prevalence area is sharply limited to southern California, Arizona, New Mexico and the western part of Texas Coccidioidomycosis is endemic in these south-western states, as evidenced by large numbers of clinical and subclinical cases of the disease arising locally From the middle of Texas and eastward across the histoplasmal endemic area, the prevalence of coccidioidin sensitivity is well below 5 per cent, and it is negligible in the north-west and south-east

In interpreting the results of testing with histoplasmin, the pattern of response as shown by the sizes of the reactions can be expected

to indicate whether specific sensitivity is involved, because it is known that reactions caused by infection with the homologous organism are generally larger than reactions caused by infection with some heterologous organism[3, 4, 5, 9].

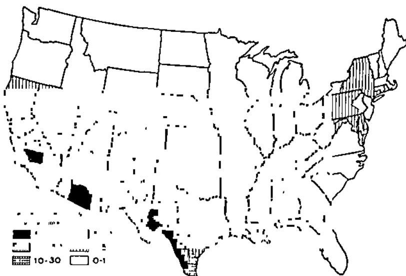


Fig 97 —Prevalence of coccidioidin sensitivity in young adults (*By courtesy of the Editor of Dis Chest*)

FREQUENCY DISTRIBUTIONS

Frequency distributions by size of reaction to histoplasmin in the histoplasmosis endemic area are given in Fig 98 The results are for recruits from 9 east-central states, in which area most of the reactions to histoplasmin can probably be ascribed to specific histoplasmal infection Groups of counties within the 9 states were combined according to the prevalence of sensitivity counties with a prevalence of more than 70 per cent were combined and called Area D, counties with from 50 to 70 per cent were called Area E, with from 30 to 50 per cent, Area F, and with from 10 to 30 per cent, Area G The sizes of the reactions for each area are plotted along the horizontal axis, and the percentages of reactions of specified sizes are *shown by the height of the corresponding column*

The frequency of reactions measuring 4 millimetres or more in Area D is about 83 per cent, in Area E about 67 per cent, in Area F 45 per cent, and in Area G 18 per cent In each instance, the reactions distribute themselves into two groups, one at the left

composed of 0, 1, 2 and some 3 and 4 millimetre reactions and the other towards the right, in a form resembling the normal probability curve, with reactions measuring from 2, 3 and 4 millimetres up to more than 25 millimetres. It can be assumed that the population in each area is composed of two groups of persons, some who have been infected with *Histoplasma* and some who have not. The two

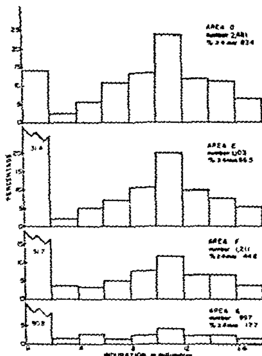


FIG 98 —Histoplasmin reactions in navy recruits from the east-central states

groups of reactions in each distribution are interpreted as representing the infected and the uninfected groups in the population Persons with 2–4 and perhaps 5 millimetre reactions, in the range where the two groups overlap, are a mixture of some infected and some uninfected Most of the infected, however, have fairly large reactions of 10–11 millimetres average diameter, and most of the uninfected have no measurable induration

Several implications may be drawn from these results about the

nature of specific histoplasmin sensitivity. First, the positive (specific) reactions are distributed fairly symmetrically around a central value, which represents the mean or average size reaction Secondly, the average size of specific reactions does not vary with the prevalence of infection, whether it is as high as 80 per cent or down to only 20 per cent. The only important difference between the four distributions is the relative proportion of large (positive) and very small (negative) reactions In the endemic area, the 5 millimetre criterion is too high for estimating the percentage of positive reactions. Many of the 5, 4 and even 3 and 2 millimetre reactions certainly belong to the positive group. The traditional 5 millimetre criterion would, if applied here, underestimate the percentage of infected in the population.

In an essentially negative population, on the other hand, the 5 millimetre criterion would overestimate the percentage of infected persons

As illustrated by the results shown in Fig 99 for lifetime residents of 5 north-western states, more than 99 per cent of the reactions *belong to the negative group at the left-hand part of the scale* Many of the 4 and 5 millimetre reactions, and even some of the 6 and 7 millimetre reactions, are interpreted as negative The very small group of large reactions centering around 10–11 millimetres probably represents the positive group, which can be most efficiently separated from the negative group at about the 7–8 millimetre level To make the customary separation arbitrarily at 5 millimetres would result, here, in overestimating, by about 100 per cent, the proportion of specific reactions, as about equal numbers of infected and uninfected would be classified as positive

A very different pattern of response is found in the south-west, where *Coccidioides* is highly prevalent Histoplasmin reactions in that area, as shown in Fig 100, form a step-like distribution from a high frequency at 0–1 millimetre to successively lower frequencies along the scale towards the larger sizes The main difference between this distribution and the one for the north-west is the relative increase in the proportion of small reactions A great many more persons living in the south-west react with 3–5 and up to 9 or 10 millimetres than in the north-west The small group of subjects showing large reactions probably represents specific sensitivity to histoplasmin, but most of the small reactions must be interpreted as non-specific sensitivity, in this instance to cross-reactions resulting from coccidioidal infection This is clearly indicated by the distributions in the lower part of Fig 100 where the total population has been subdivided into two groups those with a coccidioidin reaction of less

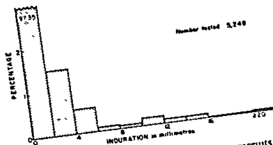
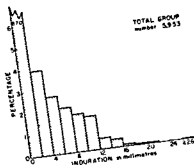


Fig 99 —Histoplasmin reactions in navy recruits from 5 north-western states



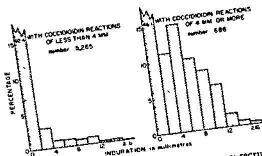


Fig 100 —Histoplasmin reactions in navy recruits from the south-western states

than 4 millimetres (left) and those with a coccidioidin reaction of 4 millimetres or more (right) Most of the small histoplasmin reactions are found in persons who *also react to coccidioidin* Simply to report that some 10 per cent of the histoplasmin reactions measure 5 millimetres or more would obscure the fact that at least two-thirds of them cannot be interpreted as specific for histoplasmal infection

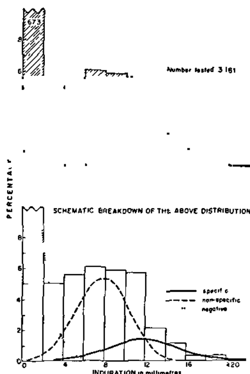


Fig 101 —Histoplasmin reactions in navy recruits from Kansas, Oklahoma, Texas and Louisiana

A somewhat similar pattern of histoplasmin reactions is found in another section of the country *Fig 101* shows the frequency distribution of reactions for lifetime residents of 4 adjacent states lying east of the coccidioidal endemic area and on the western fringe of the histoplasmal endemic area (Kansas, Oklahoma, Texas and Louisiana) Some, perhaps most, of the large reactions can be interpreted as specific for histoplasmal infection, but the small reactions filling in the range between specific and negative reactions

undoubtedly represent sensitization by some other infection. Thus, although some 30 per cent of the reactions measure 4 millimetres or more, about two-thirds of them are interpreted as non-specific They cannot, in this instance, be attributed to coccidioidal infection as the population in this area is essentially free of coccidioidin sensitivity, nor can they be ascribed to histoplasmal infection because the pattern of the distribution is so different from that found in the histoplasmal endemic area.

Another way of trying to distinguish between specific and non-specific sensitivity is by studying the relation between pulmonary calcifications and size of reactions. A high degree of correlation exists between specific histoplasmin sensitivity and pulmonary calcification[7], so if histoplasmin sensitivity is associated with a very low frequency of calcification in some area, there is good reason to believe that at least some of the reactions are not caused by histoplasmal infection. This was found to be the case in the studies made in the states referred to in Fig 101, supporting the conclusion that the bulk of the histoplasmin reactions in this zone are non-specific reactions owing to sensitization by some other agent whose identity is unknown at the present time

In Fig 102, frequency distributions are given for histoplasmin reactions in Sudan, Indonesia and India, where general population groups were tested by World Health Organization teams, using H-42 from the Public Health Service in a dilution of 1 100[1]. In Equatoria, the distribution shows the characteristic bimodal pattern, where the population clearly separates itself into two groups, one very likely infected by *Histoplasma*, the other uninfected The point of separation between the two groups might be made at about 8 millimetres In the endemic area of the United States of America, the point of separation is considerably lower, but this difference probably reflects only a difference in measuring the sizes of the reactions, for we have reason to believe that the reader in Sudan measured uniformly larger than the readers in the United States of America The important point, however, is that the pattern of the distributions in the two regions is very similar, both show two fairly distinct groups of reactions which can, with some confidence, be interpreted as representing specific and negative reactions In each case, a fair estimate can thus be made of the prevalence of histoplasmal infection in the locality In Kedisan, on the island of Bali, the small percentage of large reactions recorded may represent specific sensitivity, whereas the great mass of small reactions should probably be interpreted as non-specific, in Darjeeling, India, the population appears to consist essentially of negative reactors

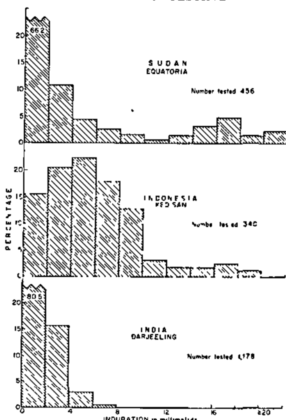


FIG 102 —Histoplasmin reactions in 3 different countries
(By courtesy of the Editor of Amer J trop Med Hyg)

ACKNOWLEDGEMENT

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RADIOLOGY OF PULMONARY MYCOSES

J. W. PIERCE

ONLY very rarely can one look at a radiograph and say that a fungous disease is responsible for the appearances seen, and even less frequently is it possible to implicate any particular fungus Like bacteria, pathogenic fungi enter the body through the respiratory tract and on reaching the lungs produce pneumonia. The shadowing which results may be of several different types It may take the form of finely scattered miliary mottling or the coarser, broncho-pneumonic type of mottling; it may present as localized denser areas of consolidation of varying size involving up to a whole lobe, and in *addition the dimorphic fungi may produce, during the stage of* invasion, a primary tubercle-like picture with shadows in the lung fields and enlarged hilar nodes. Most pathogenic fungi can produce most of these pictures, so that it is seldom possible to recognize the causative organism from the examination of a single film. The position is, however, not quite hopeless diagnostically, because at some stage in the course of these diseases most of them produce a fairly characteristic, if not absolutely typical, radiological picture.

In general the radiological appearances of fungous diseases resemble those seen in chronic tuberculosis or in the chronic suppurative pneumonias When the infection is widespread throughout the lungs the appearances usually resemble the coarser broncho-pneumonic type of mottling rather than the finer streaking and mottling seen in sarcoid or cases of diffuse interstitial fibrosis It is important to note that the time to think of a fungous disease is not when an unusual lung picture is first seen, but later, when the course of the disease and the response to treatment prove to be abnormal

ACTINOMYCOSIS AND NORTH AMERICAN BLASTOMYCOSIS

These diseases will be dealt with together because both can produce several different types of shadow and because they illustrate how confusing may be the similarity between one mycotic disease and another Fig 103 shows the widely spread broncho-pneumonic mottling in all zones in a case of actinomycosis Fig 104 comes from a case of North American blastomycosis, and in both the disease has given rise to fibrosis, displacement of the hilum and a distorted vascular pattern. These types of the two diseases are



Fig 103 —Actinomycosis, broncho-pneumonic mottling in all zones.



Fig 104 —North American blastomycosis, broncho-pneumonic mottling in all zones

virtually indistinguishable in radiographs Both diseases may also produce more dense pulmonary consolidation, sometimes on one side with smaller secondary areas of involvement on the other, and in this type the diseases are again indistinguishable There is, however, one fairly characteristic picture which sometimes occurs in actinomycosis when thickening of ribs may result from periostial new bone formation (Fig 105). Though highly characteristic, this appearance cannot be considered as absolutely conclusive since

periostitis may arise in chronic empyemata. In actinomycosis, however, rib involvement often occurs without an empyema, and may appear early in the disease.



FIG 105—Actinomycosis, periostitis of ribs

TORULOSIS

[illegible] of this disease have mostly

to involve the hilar glands At most, they may produce some obstructive pneumonia distal to the main lesion, behaving in this respect like benign tumours of the lung They may be very large and some may cavitate Torulosis may also present as scattered small rounded shadows throughout the lungs, the appearances being *indistinguishable from other causes of widespread pulmonary* mottling

ASPERGILLOSIS

At least 2 main varieties of this disease are described The most interesting is the *saprophytic* or *mycetoma* type which takes the form of a solid mass of fungus growing in a cavity These masses may be found in any pre-existing cavities, most commonly in cases of tuberculosis, or in cavitated infarcts, they do not appear to invade bronchiectatic cavities, as all the cases so far encountered have been in the upper zones This fungus does not itself appear to cause cavitation. The characteristic radiological picture of a mycetoma is

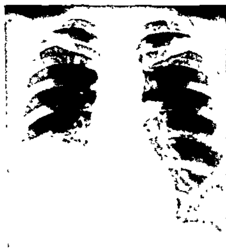


FIG 106a — Torulosis, toruloma seen as fairly well-defined lesion situated in the right middle lobe



FIG 106b — Torulosis, same case as shown in 106a, lateral view

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unattached within the cavity The persistence of such a mass in a

cavity over weeks or months is almost pathognomonic of an aspergillus mycetoma Similar appearances may be met with in a cavitating neoplasm (though it is extremely rare for cavitation to occur around the periphery of a tumour), in cases where there is a blood clot in a cavity *following a haemoptysis, or where there is a dead* shrinking hydatid cyst. These last 3 alternatives all concern dead

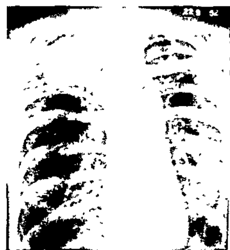


FIG 107.—Aspergillosis; mycetoma

tissues which would be expected, in time, to change and ultimately to disappear, mycetomas may persist unchanged for months and may even show gradual enlargement How they originate is not known with certainty but in the following case their development *has been observed The next figures illustrate the development of* an aspergillus mycetoma in a young female patient with well-controlled tuberculosis who suddenly began to produce sputum in profusion containing plentiful *Aspergillus fumigatus* Two very large distension cavities with fluid levels and considerable thickening of their walls were demonstrated (Fig 108a) Three months later the cavities had become smaller and it appeared that the thickened lining of one had crumpled to form a mass within the space which *remained. One year later this mass was seen to be discrete and to* have air around it (Fig 108b) This suggests that the thickened [illegible]fected wall may become displaced and may thus constitute



Fig 108a —Aspergillosis, thick-walled distension cavities (21 12 53)



Fig 108b —Aspergillosis, same case as shown in Fig 108a Mycetoma formation in right upper zone (28 1 55)

repeated episodes of fever, the expectoration of mucoid plugs, and the occurrence of transient areas of pneumonic consolidation Figure 109a shows the radiograph of a patient suffering from such an episode, 6 months later all the shadowing had cleared Two years later the shadowing returned in a different position with a small extension on the other side (Fig 109b), and after a further month additional changes were to be seen Such a clinical course coupled with these appearances, especially if associated with the expectoration of bronchial casts, is highly suggestive of this type of aspergillosis

COCCIDIOIDOMYCOSIS

During the stage of invasion this fungus may give rise to pneumonia producing areas of consolidation in the lung, the shadows resembling those seen in any ordinary bacterial or viral pneumonia In cases where the infection is overwhelming, the disease may spread as a miliary mottling throughout both lungs In addition, the initial infection may closely simulate primary tuberculosis with discrete foci in the lung and enlarged hilar glands, both of which may later calcify.

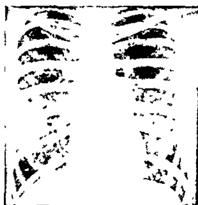


Fig 109a —Aspergillosis, allergic type Areas of pneumonic consolidation (2 1 53)



Fig 109b —Aspergillosis, same case as shown in Fig 109a, new areas of consolidation (12 10 56)

The course of the disease is usually benign, the shadows clearing and leaving, at most, small calcified foci An occasional area of consolidation may cavitate, or may partially resolve, leaving behind a few isolated rounded opacities which are indistinguishable from those seen in pulmonary tuberculosis, and which may be visible for years Sometimes a cavity will remain after the surrounding consolidation has cleared, and may persist for many months or years as a smooth thin-walled cavity, usually without fluid, and liable to changes in size with alteration in tension (Fig 110) This appearance is highly suggestive of coccidioidomycosis, and is almost diagnostic in persons living in the endemic area.

HISTOPLASMOSIS

Histoplasmosis may also occur in several different forms The disease may present with one or more areas of pneumonia (which unlike those of coccidioidomycosis do not cavitate), with an isolated lung lesion and enlarged glands as in primary tuberculosis, or with widespread small foci densely scattered throughout both lungs

The initial stage of invasion usually heals without permanent lung damage, but both glands and lung lesions are very liable to calcify

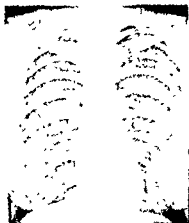


FIG 110 —Coccidioidomycosis, thin-walled cavity

(Figs 111a and 111b) These rather dense, regular, rounded opacities are the most characteristic appearance of histoplasmosis, but they are not easily distinguishable from those found in tuberculosis On the whole, they are a little more densely calcified, tend to remain discrete with less evidence of aggregation, and to be more evenly scattered over the lung fields without the predominantly upper zone distribution of the calcified foci of tuberculosis

This fungus can also produce a form of chronic fibrosing lung disease which can exactly mimic chronic pulmonary tuberculosis, even to progressive fibrosis and cavitation (Figs 112a and 112b)

The only 2 cases of acute histoplasmosis found in this country were both in patients who had lived in endemic areas, though both had been in England for 5 years Both patients died from the acute disseminated form of the disease, the spread in both cases following shortly on a biopsy, in one case of a laryngeal lesion thought to be tuberculous, the other of a tonsillar ulcer

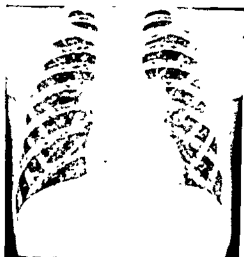


FIG 111a — Histoplasmosis; widespread patchy consolidation and glandular enlargement

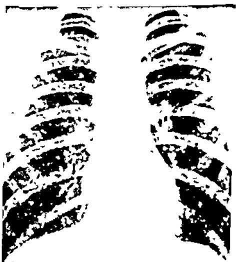


FIG 111b — Histoplasmosis, same case as shown in Fig 111a, 4 years later Calcified nodules

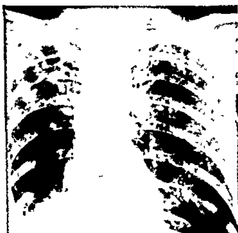


Fig 112a —Histoplasmosis, lesions closely resembling chronic pulmonary tuberculosis

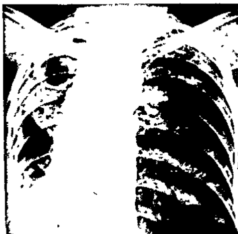


Fig 112b —Histoplasmosis, same case as shown in Fig 112a, 5 years later Cavitation and fibrosis on right side

PART II

TREATMENT OF MYCOTIC DISEASES

THE MODE OF ACTION OF ANTI-FUNGAL DRUGS

GORDON T. STEWART*

In this study 5 groups of drugs were compared in mode of action *in vitro* against 5 groups of fungi The drugs, 10 in number, represented widely differing substances: polyenes, diamidines, quinolines, aliphatic substances and triphenylmethane dyes The organisms were from the main pathogenic groups, at least 2 different varieties of each genus being represented

RESULTS

These substances varied considerably in activity (Table 40). The widest range was shown by 2 chlorinated oxyquinolines, which were strongly inhibitory to all the organisms tested at concentrations of 10 µg per millilitre or lower. The highest degree of activity against any one organism was that of amphotericin B against *Candida albicans* and *Microsporum audouini*, and of pentamidine against *Aspergillus niger*. Activity of this order (1 µg. per millilitre) is fairly specific, and is not part of a general anti-protoplasmic toxicity.

The components of activity shown by these various drugs were as follows

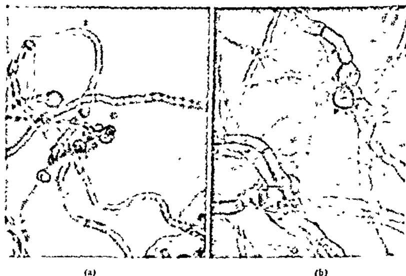
DIRECT EFFECTS UPON THE MORPHOLOGY OF THE ORGANISMS

This was studied by microscopy of living cells from cultures containing the various drugs. It is technically important, in direct comparisons of this kind, to use the drugs in soluble forms but, at the same time, to exclude any independent or adjuvant effects contributed by the solvents In our experiments, the solvent (dimethyl formamide) was present at a final strength of 1 per cent in all preparations, control and test, at which strength it appeared to be quite inert

Direct microscopic observation of living, unstained preparations showed that none of the drugs possessed an immediate, cytotoxic effect The earliest visible effect was the inhibition of budding in the yeast-like organisms in 3–8-hour cultures, best shown by the polyene antibiotics and the halogenated oxyquinolines Thereafter, abnormal morphological changes gradually began to occur in hyphae and spores, but only with certain drugs

* With the technical assistance of R J Holt

Hyphae.—The two halogenated oxyquinolines at 2 µg. per millilitre caused swelling and vacuolation in cultures of *A. niger* (Fig 113) The hyphae were especially thin and rarefied where conidia were forming This type of change was obvious after 24–48 hours' growth. Rimocidin (10 µg. per millilitre; Davisson and his colleagues[2]) caused similar though less obvious changes, and also affected visibly the hyphae of *Aspergillus fumigatus* and *Trichophyton mentagrophytes*.



(a) (b)

FIG 113 —*Aspergillus niger* (a) Normal hyphae (b) Effect of chlorhydroxyquinoline (5 µg per millilitre), swelling and vacuolation of hyphae (× 360)

Spores.—Rimocidin (5 µg per millilitre) began to act upon *C albicans* in 4 hours At 8 hours, the majority of the spores showed slight swelling and vacuolation and, by 24 hours, this change was very marked indeed When studied by interference microscopy (Fig 114), the changes in the refractility and other optical properties of the cells were very striking This rimocidin effect was present even while a proportion of the cells were budding, so it obviously did not affect all phases of growth Nystatin, in contrast, caused an absolute cessation of budding without causing comparable cytoplasmic changes, though some shrinkage and loss of refractility were evident at 8 hours This recalls the observation of Blank[3] who found by electron microscopy that nystatin caused intracellular lysis slowly, in 3–8 days, without damaging the cell wall

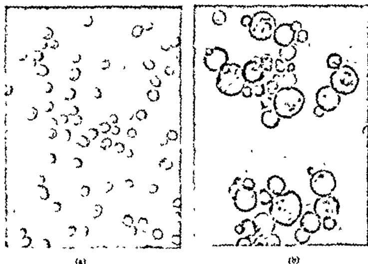


FIG 114.—*Candida albicans* (a) Normal cells (b) Effect of rimodicin (2 μg per millilitre); swollen cells with abnormal vacuolation and granulation of cytoplasm (× 360)

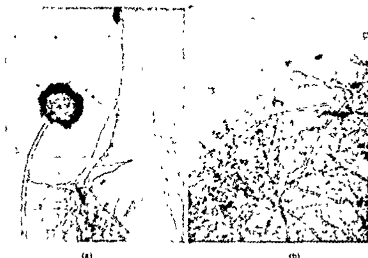
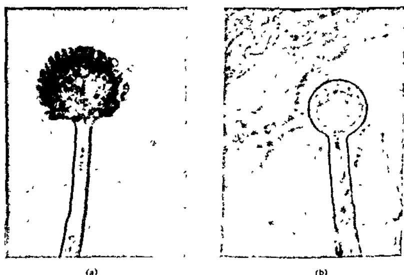


FIG 115.—*Aspergillus niger* (a) Normal conidiophores and hyphae (b) Effect of chlorhydroxyquinoline (5 μg per millilitre), abnormal conidiophores, defective sporulation (× 90)

Another conspicuous effect was that of the halogenated oxyquinolines upon the sporing of *A. niger*. The vesicles developed, but were almost devoid of mature spores or pigment and "bald" in appearance (Figs 115, 116).

EFFECTS UPON CELL DIVISION

The polyene antibiotics, pentamidine and the halogenated oxyquinolines were all readily fungistatic, in that complete inhibition of cell division could usually be attained at levels of not more than



(a) (b)

FIG 116—*Aspergillus niger* (a) Normal sporulation (b) Effect of chlorhydroxyquinoline, normal spore-bearing vesicle with absence of spores (×360)

20 μg per millilitre (Table 40), which should be attainable therapeutically in many types of lesion There were, however, some important exceptions

Nystatin, in spite of its profound effect upon *Candida* and *Microsporum* species, was much less active towards species of *Aspergillus* and *Trichophyton*, whereas amphotericin B (also a polyene) was highly active against these organisms, suggesting that the hexaenic or heptaenic form conferred greater activity than the diene-tetraene structure of nystatin None of the 3 antibiotics was active against the actinomycetes, which is not surprising in view of their origin from the *Streptomyces* species, but the oxyquinolines and pentamidine were fully active In general terms, higher concentrations of

TABLE 40

EFFECT OF VARIOUS GROUPS OF DRUGS *IN VITRO* UPON REPRESENTATIVE PATHOGENIC FUNGI

Group of drugs	Name of drug	Minimum fungistatic concentration in μg per millilitre against				
		Candida C albicans	*Actinomycetes N asteroides*	*Aspergillus A niger*	*Trichophyton T mentagrophytes*	*Microsporum M audouini*
Polyene antibiotics	Nystatin	10	≫20	>20	>20	2
$(CH{=}CH)_n$	Amphotericin B	1	>20	20	5	1
Other antibiotics	Rimocidin	15	≫20	5	10	10
Diamidines (HN, H_2N ... $-O[CH_2]_n-O-$ ... NH, NH_2)	Pentamidine	20	10	1	≫20	2
Quinolines (Halogenated)	Chlorhydroxyquinoline	5	5	5	10	2
	Dichlorhydroxyquinaldine	5	5	10	10	1
Aliphatic alcohols and acids	n-Propanol	600		12,000		
CH_3 / CH_2CH $[CH_2]_n$ {OH / COOH}	Undecylenic acid	(100)	>20	≫20	≫20	2
Triphenylmethanes (Me_2N ... $C=$ ... NMe_2, Me_2N)	Gentian violet	(100)	≫20	≫20	≫20	20

all the drugs were required to inhibit the growth of *Aspergillus* and *Trichophyton* than that of *Microsporum* or *Candida*. Organisms growing at subinhibitory concentrations of these substances were successively subcultured into media containing rising levels of the same substances, without showing intensification of growth It seemed that, in spite of the lack of lysis, drug-resistance *in vitro* was not easily acquired.

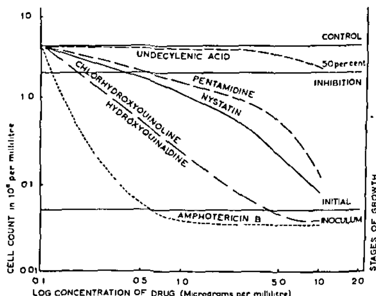


FIG 117 —The effect of various drugs upon the growth of *Candida albicans in vitro*

FUNGICIDAL EFFECTS

Interference with growth, and fungistasis, were relatively easily obtained *in vitro* Fungicidal effects (lysis of inoculum and sterile subculture) were rare (Fig 117) Even cells which had been greatly altered in appearance by rimocidin or the oxyquinolines seemed able to revive and propagate normally in drug-free subcultures Amphotericin B was the only drug which lowered the blastospore count and actually killed the bulk of the inoculum of *Candida*. The oxyquinolines, pentamidine and amphotericin were partly lethal to *A. niger* and *A fumigatus*, and the oxyquinolines had some destructive action upon *T mentagrophytes*.

METABOLIC INTERFERENCE

Carbohydrates are key substances in the metabolism of pathogenic fungi Thus laevulose, dextrose and maltose greatly enhance, in that order, the growth of *Candida*. There are a number of points of interaction between the carbohydrate metabolism of fungi and their inhibition by drugs. The polyenes were found to be 20–40 times more active against *Candida* in laevulose than in dextrose (Fig 118) whereas the oxyquinolines and pentamidine were correspondingly more active in dextrose than in laevulose This was

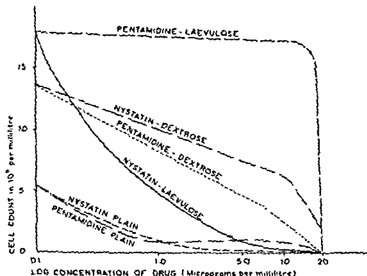


FIG 118 —The effect of nystatin and pentamidine upon *Candida albicans* in plain peptone water, d(+) dextrose peptone water, and d(–) laevulose peptone water

independent of *p*H change, and no such interactions were exhibited by these sugars against undecylenic acid, rimocidin or gentian violet A similar, antagonistic effect was shown by dextrose against the action of the polyenes on *A niger*

The difference in the interaction of dextrose and laevulose suggested that isomerism might be a factor This was borne out by the fact that *n*-propanol was much more active than its isomer against *Saccharomyces*, *Candida* and *Aspergillus* (Fig 119) Such inhibitory action on the part of alcohols was influenced also by aliphatic-chain length, as ethanol, propanol and butanol were

THE THERAPEUTIC USE OF ANTIFUNGAL AGENTS

E. DROUHET

THE fungous diseases, often chronic and sometimes fatal, are among the most difficult infections to control. Bacterial infections can be controlled by vaccines, antisera and numerous antibacterial substances Viral infections, though lacking specific treatment, can be prevented by immunization These measures are not available, however, for the mycotic infections.

Numerous antifungal agents are active *in vitro*, but few are effective *in vivo*. The great majority of chemical antifungal agents are effective only in the superficial mycoses by local applications; few can be used by the oral route (Table 41)[7, 36, 38, 39, 43, 45].

ANTIFUNGAL DIAMIDINES AND ANTIBIOTICS

The aromatic diamidines are effective by oral or parenteral routes in the systemic blastomycoses, but toxic side-effects limit their use More promising than synthetic chemical agents for the treatment of fungous diseases are the antifungal antibiotics The various antibiotics so far isolated may be divided after Waksman[60] into 3 broad groups on the basis of their respective antimicrobial spectra (Table 42) Besides the antibiotics with antibacterial and anti-actinomycotic spectra there is another category of antibiotics (including tyrothricin and gliotoxin) which is active both on bacteria and fungi but too toxic for therapeutic use, the last and most interesting category for fungous treatment includes antibiotics which are selectively antifungal such as actidione (cycloheximide) and the polyenes $(CH=CH)_n$.

Actidione.—This is highly active *in vitro* on one pathogenic fungus only, *Cryptococcus neoformans* In animals, actidione affords no protection to mice infected with this yeast, but in humans[40] it has some activity by intrathecal, intravenous and intramuscular routes (Table 48).

Polyenes.—The most interesting antifungal antibiotics are the polyenes, products of *Streptomyces*, possessing in common a conjugated polyene chromophore[48]. They appear to possess generally similar chemical properties but can be classified by examination of their ultraviolet absorption spectra into 4 subgroups, corresponding to the presence of 4, 5, 6 or 7 conjugating double bonds

TABLE 41

CHEMICAL STRUCTURE AND THERAPEUTICAL USE OF SOME PRINCIPAL ANTIFUNGAL AGENTS

Chemical antifungal agents	*Therapeutic use*	
	Route of administration	*Fungous diseases*
Heavy metals: Hg, Ag, Cu, Zn (salts)	Local	Superficial mycoses
Halogens: I(1), Br, Cl	Local (1) Oral or parenteral	Superficial mycoses (1) Sporotrichosis
Sulphur compounds dithiocarbamate (2); rhodanine (3); tetraethylthiuram disulphide (4)	(4) Local	(4) Superficial mycoses
Fatty acids $CH_2{=}CH{-}(CH_2)_8{-}COOH$ undecylenic acid (5) $CH_3(CH_2)_6COOH$ caprylic acid (6) $CH_3{-}CH_2{-}COOH$ propionic acid (7)	(5) (6) Local (5) (6) Oral (7) Local	(5) (6) Superficial mycoses (5) (6) Digestive moniliasis (7) Superficial mycoses
Quaternary ammonium compounds $[R_1R_2R_3R_4N]^+ X^-$ (8)	(8) Local	Superficial mycoses
Antihistamines chlorocyclizine HCl methaphenilene HCl diphenhydramine HCl, etc	Local	Dermatomycoses
Compounds with Benzoic Nucleus Benzoic derivatives benzoic acid (9); salicylic acid (10); *p*-hydroxybenzoic acid (11); ethyl vanillate (12)	(9) (10) Local (11) (12) Oral	(9) (10) Superficial mycoses (11) Digestive moniliasis (12) Histoplasmosis

TABLE 41 (*continued*)

Chemical antifungal agents	*Therapeutic use*	
	Route of administration	*Fungous diseases*
Sulphonamides, sulphones		
sulphanilamide (13) (NH_2 / SO_3NH_4); p amino benzoic acid (14) (NH_2 / COOH); diaminodiphenyl sulphone (15) (SO_2, NH_2, NH_2)	(13) Oral (15) Oral	(13) Anaerobic actinomycosis, South American blastomycosis, (15) Nocardiosis
Phenol derivatives		
phenol (16) (OH); cresol (17) (CH_3 / OH); pentaethylene glycolic ether of dichlorocresol (18)	(18) Local	Superficial mycoses
Benzothiazoles 2-dimethyl-amino-6-(β diethyl-amino-ethoxy) benzothiazol (19)	(19) Local	Superficial mycoses
Dyes: Triphenylmethane group		
$[(CH_3)N-C_6H_4-C(C_6H_4-N(CH_3)_2)(C_6H_5)]Cl$ malachite green (20)	(20) Local	Superficial mycoses
Quinones		
tetrachloropara-benzoquinone (21) (Cl Cl / Cl Cl); 2-methoxy-1,4-naphthoquinone (22) ($-OCH_3$)	(21) (22) Local	Dermatomycoses

TABLE 41 (*continued*)

Chemical antifungal agents	*Therapeutic uses*	
	Route of administration	*Fungous diseases*
Quinolines		
5,7-dichloro-8-hydroxy-quinoline (23); 5-chloro-7-iodo-8-hydroxy-quinoline (24)	(23) Oral (24) Local	(23) Digestive moniliasis (24) Superficial mycoses
Aromatic diamidines		
(25)	(25) Oral and perfusion	North American blastomycosis Coccidioido-mycosis

Nystatin, Candicidin and Euclin.—The comparative fungal spectra of various antifungal antibiotics are given in Table 43 Nystatin, a tetraene polyene antibiotic discovered by Hazen and Brown[27] in [illegible]

as *C neoformans*, *Blastomyces dermatitidis* and *Histoplasma capsulatum* Amphotericin B (a heptaene) is particularly active on deep-seated fungi[22, 53, 59] Euclin, a new antibiotic of unknown composition, inactive against *Candida*, is highly inhibitory to fungi causing the deep-seated mycoses, as well as on some bacteria and actinomycetes[62]

Nystatin in animal studies and human therapy.—Data on the toxicity of the antifungal antibiotics is summarized in Table 44 while Tables 45 and 46 show their therapeutic activity in experimental mycoses. The numerous studies on various animals show a definite activity and low toxicity of nystatin by the oral route and very favourable activity in systemic mycoses by the parenteral route but not by the oral route, due to poor absorption[1, 3, 15, 23, 31]

The first antifungal antibiotic that found a large application in human therapy was nystatin. The need to control post-antibiotic complications due to *Candida* was satisfied by this antibiotic, even

TABLE 42
A CLASSIFICATION OF THE PRINCIPAL ANTIBIOTICS

Antibiotic activity	*Antibiotics*	*Producing organism*
Antibacterial and anti-actinomycotic	Penicillin	*Penicillium notatum, P. glaucum*
	Streptomycin	*Streptomyces griseus*
	Chloramphenicol	*S. venezuelae*
	Chlortetracycline	*S. aureofaciens*
	Oxytetracycline	*S. rimosus*
	Neomycin	*S. fradiae*
Antibacterial and Antifungal	Tyrothricin	*Bacillus brevis*
	Thiolutin	*Streptomyces albus*
	Clavacin	*Aspergillus clavatus*
	Streptothricin	*S. lavendulae*
	Actinomycin	*S. antibioticus*
	Gliotoxin	*Trichoderma*
	Trichothecin	*Trichothecium roseum*
Antifungal	Cycloheximide (actidione)	*Streptomyces griseus, S. noursei*
	Polyenes:	
	Tetraenes	
	Nystatin	*S. noursei*
	Rimocydin	*S. rimosus*
	Antimycoin	*S. aureus*
	Chromin	*S. antibioticus*
	Amphotericin A	*S. sp.*
	Pentaenes	
	Eurocidin	*S. sp.*
	Fungichromin	*S. sp.*
	Fungichromatin	*S. sp.*
	Filipin	*S. filipinensis*
	Hexaenes	
	Flavacide	*S. sp.*
	Mediocidin	*S. sp.*
	Heptaenes	
	Candicidin	*S. griseus*
	Candimycin	*S. griseus*
	Ascosin	*S. canescus*
	Trichomycin	*S. hachijoensis*
	Amphotericin B	*S. sp.*

in infants and children in whom blood-borne dissemination of *C. albicans* from intestinal capillaries[11], or by extension from the bronchi and the lungs[35], may be particularly dangerous. It also controls chronic cases of onychia due to *C. albicans* resistant to ordinary treatment[26]. Table 47 summarizes the results with nystatin in the hands of various authors[12, 13, 14].

[illegible] of nystatin in 46 cases of [illegible] laboratory [illegible] were cases

TABLE 43
COMPARATIVE SPECTRA OF VARIOUS ANTIFUNGAL ANTIBIOTICS

Test organism		Minimum inhibitory concentration in µg/ml of*						
			Polyene antibiotics					
		Cycloheximide Actidione	Nystatin Tetraene	Candicidin Heptaene	Trichomycin Heptaene	Amphotericin A Tetraene	Amphotericin B Heptaene	Euclin
	Candida albicans	>1 000	3·12	2·5	0·7	4·7	3·7	120·8
	C. tropicalis		3·12	5·0	0·7	4·7	>40	
	Geotrichum	>1 000	6·25	2·5	0·3	12·5	>40	
Deep fungi	*Cryptococcus neoformans*	0·24	1·56	1	0·17	3·1	0·6	0·07
	Sporotrichum schenckii (Y)	>1 000	12·5	200	50	3·9	0·14	
	Blastomyces dermatitidis (Y)	>1 000	1·56	1	0·3	1·2	0·6	0·03
	Histoplasma capsulatum (Y)	>1 000	1·56	1	0·7	0·8	0·04	0·07
	Coccidioides immitis	>1 000	1·56	500			+	
Dermatophytes	*Trichophyton mentagrophytes*	>1 000	12·5	500	50	9·4	14	2·3
	T. tonsurans		6·25	500		5·5	>40	
	T. rubrum	>1 000	12·5	500		4·7	30	
	Microsporum audouini		6·25	500		1·2	1·9	
	M. canis		6·25	500	0·3	9·4	30	
	M. gypseum		2·5			2·5	>40	9·5
	Epidermophyton floccosum		3·12	500	0·3	1·6	0·9	1·2
Subcutaneous fungi	*Monosporium apiospermum*	25	200			6·3	>40	0·03
	Cladosporium werneckii					6·3	>40	0·07
	Phialophora verrucosa	12·5	12·5			4	>40	0·28
	Aspergillus fumigatus	400	3·12		>50	6·3	>40	
	Penicillium sp	100	13					
	Actinomycetes	0	0	0	0	0	0	*N. asteroides* 2·3
	Bacteria	0	0	0	0	0	0	± 0·7–14

* M.I.C. for 4 days established in agar medium except for Trichomycin and Euclin
(Y)=Yeast phase

TABLE 44

TOXICITY OF SOME ANTIFUNGAL ANTIBIOTICS ON ANIMALS

Antibiotics	*Chemical nature*		*Acute toxicity, LD 50 in mg/kg.* — *Route of administration*: *Oral*	*Sub-cutaneous*	*Intra-peritoneal*	*Intra-venous*
Nystatin	Polyenes	Tetraene	3,000 mg (800,000 U)	150 mg	20–26 mg (30,000 U)	
Trichomycin	Polyenes	Heptaenes	250–1,000 mg	17 9 mg (4,300 U)	2 2 mg (4,200 U)	2 2 mg (4,300 U)
Amphotericin B	Polyenes	Heptaenes	>280 mg	280 mg		20 mg
Ascosin	Polyenes	Heptaenes	500 mg	168 mg	8 6 mg	12 5 mg
Candicidin	Polyenes	Heptaenes			14 mg	
Antibiotic 1968	Polyenes	Heptaenes	>500 mg			250 mg
Actidione	Cyclo-heximide			110 mg (cumulative toxic effect)	138 mg (cumulative toxic effect)	
Euclin			430 mg	153 mg	130 mg	

of generalized moniliasis with thrush, diarrhoea, vulvo-vaginitis and multiple abscesses, and 25 were cases of digestive moniliasis A rapid clinical recovery was obtained in all cases and *Candida* decreased in numbers, or disappeared completely, from the mouth, stool, urine or blood when nystatin was administered in powder form Daily doses of nystatin can reach or exceed $1\frac{1}{2}$ million units for infants, 2 to 3 million units for children and 6 to 10 million units for adults, and can be continued at this level for many days There is a remarkable lack of toxicity The powdered substance is the most adequate preparation for the treatment of digestive moniliasis, because generally the whole gastro-intestinal tract is involved in such cases, beginning at the oral cavity Clinical relapses occurred in several cases, particularly where thrush was unrelated to antibiotic therapy. No resistant strains of *C. albicans* were encountered In

TABLE 45

THERAPEUTIC ACTIVITY OF NYSTATIN IN EXPERIMENTAL MYCOSES

Experimental mycosis	*Route of administration*	*Experimental animals*	*Therapeutic effect*	*References*
Systemic moniliasis	Parenteral	Mice	Favourable	5 6 27 44 56
Systemic moniliasis	Parenteral	Rabbits	Favourable	35
Systemic moniliasis	Parenteral	Embryonated eggs	Favourable	53
Systemic moniliasis	Oral	Rabbits	No effect	33
Digestive moniliasis	Oral	Mice	Favourable	34
Digestive moniliasis	Oral	Rabbits	Favourable	14 27 28
Digestive moniliasis	Oral	Dog	Favourable	30
Dermatophytosis	Local	Guinea-pig	Favourable	46
Systemic cryptococcosis	Parenteral	Mice	Favourable	8 27 28
Systemic coccidioidomycosis	Parenteral	Mice	Favourable	6 46
Systemic sporotrichosis	Parenteral	Mice, hamsters	Fair	6 42
Blastomycosis	Parenteral	Mice	Favourable	28
Blastomycosis	Oral	Mice, hamsters	No effect	18
Histoplasmosis	Parenteral	Mice	Favourable	8 28
Histoplasmosis	Parenteral	Mice, hamsters	Favourable	17

localized broncho-moniliasis of adults, results of oral nystatin are not always satisfactory because of the poor absorption of this antibiotic, as Stewart[57] demonstrated

In vaginitis, good results were obtained in many trials[4, 14, 15, 25, 26, 32], even when several relapses occurred These relapses, which are usually seen in pregnant women and are often due to an intestinal focus or to reinfection by the husband, were cured by a second course of treatment

In cutaneous moniliasis, the topical application of nystatin is very effective[34], even in cases of chronic onychia Grupper[26] from Hôpital Saint Louis obtained the best results by combining topical nystatin applications with Sabouraud's procedure of removing all infected foci from the bases of the nails to allow penetration of the antibiotic to the deepest layers of keratin In chronic cases of moniliasis, intensive search is advisable to establish primary conditions (endocrine factors, avitaminosis, and so on) predisposing to fungal infection and sometimes responsible for resistance to antifungal therapy

Response to nystatin was offered as proof that *C albicans* might be responsible for angiocholecystitis by Crismer and his colleagues[10],

TABLE 46
THERAPEUTIC ACTIVITY OF SOME ANTIFUNGAL ANTIBIOTICS IN EXPERIMENTAL MYCOSES

Antibiotics	*Experimental mycoses*	*Route of administration*	*Animals*	*Therapeutic effect*	*References*
Candicidin	Digestive moniliasis	Oral	Mice	Favourable	33
	Systemic moniliasis	Parenteral	Mice	Favourable	
	Blastomycosis	Parenteral	Mice	Favourable	
	Sporotrichosis	Parenteral	Mice	Favourable	
	Histoplasmosis	Parenteral	Mice	Fair	
	Cryptococcosis	Parenteral	Mice	Fair	
Trichomycin	Moniliasis	Oral or parenteral	Mice, rats, rabbits	Favourable	30
	Trichophytosis	Local	Guinea-pig	Favourable	
Ascosin	Moniliasis	Parenteral	Mice	Favourable	29
	Histoplasmosis	Parenteral	Mice	Favourable	19
Antibiotic 1968	Systemic moniliasis	Parenteral	Mice	Favourable	6
	Sporotrichosis	*Parenteral*	*Mice*	*Favourable*	
	Histoplasmosis	Parenteral	Mice	Favourable	
Euclin	Blastomycosis	Oral or parenteral	Mice	Favourable	62
Amphotericin A	Moniliasis	Oral	Mice	No effect	33
	Moniliasis	Parenteral	Mice	Favourable	
	Histoplasmosis	Oral	Mice	Fair	
	Blastomycosis	Oral	Mice, hamsters	No effect	
Amphotericin B	Moniliasis	Oral	Mice	Favourable	53
	Moniliasis	Parenteral	Mice	Favourable	55, 56
	Coccidioidomycosis	Oral or parenteral	Mice	Favourable	56
	Cryptococcosis	Oral or parenteral	Mice	Favourable	41, 53
	Histoplasmosis	Oral	Mice	Favourable	41, 53
	Histoplasmosis	Parenteral	Mice, hamsters	Favourable	17
	African histoplasmosis	Oral	Mice, hamsters	Favourable	16
	Blastomycosis	Oral	Mice, hamsters	Favourable	18

who observed 2 cases, with *C albicans* in the bile, in whom persistent fever for several months was rapidly controlled by nystatin

Nystatin in prophylaxis—Prophylactic administration of nystatin prevented all cases of thrush in the Centre for Premature Infants in Paris[35] over the last 2 years, in abdominal surgery nystatin combined with different antibacterial agents has been used for sterilization of the bowel[52, 55].

TABLE 47
THERAPEUTIC USE OF NYSTATIN IN HUMAN MONILIASIS

Clinical form of moniliasis (candidiasis)	Number of cases	Age-group I = Infants C = Children A = Adults	Route of administration	Doses of Nystatin		Results E = excellent G = good R = relapse F = Failure	References
				daily in millions U.	period d = days w = weeks m = months		
Generalized	13	3I, 7C, 3A	Oral	0·5–1·5	4–8d	13E, 1R	13
Digestive	25	20I, 3C, 2A	Oral	0·5–1·5	2–8d	25E, 7R	13
Septicaemic	1	A	Intravenous	0·4	6d	E	49
Oral	49		Oral	0·5–2	3d–3m	19E, 29G, 1F	64
Vaginal	17	A	Local	0·1	1–6w	9E, 8G	
Cutaneous	63		Local	0·1	1–12w	25E, 34G, 4F	46
Onychia	39	A	Local + nail treatment	0·1	1–4m	32E, 5G, 2F	
Mucous	14	A	Local + oral	0·1–1·5	3–15d	11E, 3G, 1R	
Mucocutaneous	17	6C, 11A	Local	0·1–1·5	3–35d	13E, 3G, 1F	26
Generalized cutaneous	8	C, A	Local + oral + cortisone	1–5	3–80d	7E, 1G	
Onychia	19	A	Local	0·1	2–6w	9G, 10F	
Generalized cutaneous	3	2C, 1A	Oral	0·5–1		3G	
Oral	4	C	Oral	0·4	1w	4G	51
Vulvo-vaginitis	20	A	Local			20G	
Brain abscess	1	A	Intramuscular	0·4	10d	F	
Pharyngitis + bronchitis	12	A	Oral	1–2	7–10d	9E, 2G, 1F	57
Oral	8	2C, 6A	Oral			7E, 1F	2
Vagina	20	A	Local + oral	0·2	5d	20E	4
Vaginal	58	A	Local	0·1		58E, 11R	32
Angiocholitis	2	A	Oral	3–5	6d	2E	30
Abdominal	2	A	Oral	2	1w	2E	47

TABLE 48
THERAPEUTIC USE OF SOME ANTIFUNGAL AGENTS IN CLINICAL CASES OF MYCOSES

Antibiotics	*Fungal disease*	*Number of cases*	*Route of administration*	*Results* E=*excellent* G=*good* f=*fair* F=*failure*	*References*
Nystatin	Dermatophytosis (60 *Trichophyton rubrum*, 26 *T mentagrophytes*, 7 other)	93	Local	4E, 7G, 48f, 34F	24
	Coccidioidomycosis	10	Oral	1G, 9F	46
		2	Intramuscular	2F	46
		5	Intravenous	2G, 3F	46
		1	Intravenous	F	
	Histoplasmosis	2	Oral	2F	
		4	Intravenous	4F	38,39
	Cryptococcosis	2	Oral	2F	40
		2	Intravenous	2F	38,39
	Brain abscesses with *Cladosporium trichoides*	1	Oral and intramuscular	F	
Candicidin	Moniliasis				
	Cutaneous	I	Local	E	20
	Vaginal	1	Local	E	21
Trichomycin	Moniliasis				
	Cutaneous		Local	E	30
	Vaginal		Local	E	9
	Digestive		Oral	E	30
Ascosin	Tinea capitis	102	Local	53G, 49F	
Actidione	Cryptococcosis	19	Intravenous, intramuscular and intrathecal	2G, 4f, 13F	40
Amphotericin B	Histoplasmosis	1	Oral	G	38,39

Conclusion.—In the mycoses other than moniliasis, the results with nystatin are unsatisfactory (Table 48) In dermatomycosis, there is no favourable response[24], and, in the systemic mycoses, the drug is ineffective when given orally because of poor absorption Local reaction limits its use intramuscularly By the intravenous route (200,000–400,000 units in 6 hours perfusion daily) a good blood level of antibiotic is obtained but high fever and chills limit its use to very severe infections[46]

Other antifungal antibiotics have found little application in clinical cases, as is shown in Table 48.

Experimental studies in animals with amphotericin B by oral or parenteral routes have shown remarkable activity in the hands of several workers (Table 46)[41, 53, 56], notably in the blastomycosis of hamsters and of mice[18] In our experiments 100 per cent of control animals were dead after 3 weeks as well as the animals treated with

TABLE 49

RESULTS OF CULTURES FROM THE LIVER, SPLEEN AND LUNGS OF ANIMALS INFECTED WITH *BLASTOMYCES DERMATIDITIS* AND TREATED WITH ANTIBIOTICS

Treatment	*Per cent positive cultures* (0 20 40 60 80 100 per cent)		
Control or Amphotericin A	++++++++++++++++++++	Liver	Hamsters
	++++++++++++++++++++	Spleen	
	++++++++++++++++++++	Lungs	
Nystatin	++++++++++++++	Liver	
	++++++++	Spleen	
	++++++++++++	Lungs	
Amphotericin B or Amphotericin AB	0	Liver	
	0	Spleen	
	0	Lungs	
Control or Amphotericin A	++++++++++++++++++++	Liver	Mice
	++++++++++++++++++++	Spleen	
	++++++++++++++++++++	Lungs	
Nystatin	++++++++++++++++++++	Liver	
	++++++++++++++	Spleen	
	++++++++++++++++++++	Lungs	
Amphotericin B or Amphotericin AB	0	Liver	
	0	Spleen	
	0	Lungs	

nystatin or with amphotericin A. All animals treated with amphotericin B remained alive at the end of this experiment, no macroscopic lesions were observed and cultures of spleen, liver and lungs were negative (Table 49)

In African histoplasmosis we obtained similar good results[16] Preliminary clinical results with oral and intravenous amphotericin B in cryptococcal meningitis and in disseminated histoplasmosis reported by Lehan and his colleagues[38, 39] permit us to hope for the eventual control of systemic and deep mycoses.

TABLE 48

THERAPEUTIC USE OF SOME ANTIFUNGAL AGENTS IN CLINICAL CASES OF MYCOSES

Antibiotics	*Fungal disease*	*Number of cases*	*Route of administration*	*Results* E=*excellent* G=*good* f=*fair* F=*failure*	*References*
	Dermatophytosis (60 *Trichophyton rubrum*, 26 *T mentagrophytes*, 7 *other*)	93	Local	4E, 7G, 48f, 34F	24
	Coccidioidomycosis	10	Oral	1G, 9F	46
		2	Intramuscular	2F	46
Nystatin		5	Intravenous	2G, 3F	46
		1	*Intravenous*	F	
	Histoplasmosis	2	Oral	2F	
		4	Intravenous	4F	38,39
	Cryptococcosis	2	Oral	2F	40
		2	Intravenous	2F	38,39
	Brain abscesses with *Cladosporium trichoides*	1	Oral and intramuscular	F	
	Moniliasis				
Candicidin	Cutaneous	1	Local	E	20
	Vaginal	1	Local	E	21
	Moniliasis				
Trichomycin	Cutaneous		Local	E	30
	Vaginal		Local	E	9
	Digestive		Oral	E	30
Ascosin	Tinea capitis	102	Local	53G, 49F	
Actidione	Cryptococcosis	19	Intravenous, intramuscular and intrathecal	2G, 4f, 13F	40
Amphotericin B	Histoplasmosis	1	Oral	G	38,39

Conclusion.—In the mycoses other than moniliasis, the results with nystatin are unsatisfactory (Table 48) In dermatomycosis, there is no favourable response[24], and, in the systemic mycoses. the drug is ineffective when given orally because of poor absorption Local reaction limits its use intramuscularly By the intravenous route (200,000–400,000 units in 6 hours perfusion daily) a good blood level of antibiotic is obtained but high fever and chills limit its use to very severe infections[46].

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The following approaches to treatment of ringworm require consideration·

DISTURBANCE OF THE HOST-PARASITE BALANCE

Inflammatory reactions, particularly in follicle infections, are essentially curative. Eczematous reactions which may occur when a patient becomes sensitive to a fungicide applied locally are not in any way curative, and can only be regarded as an attempt on the part of the patient to rid himself of the fungicide and not the fungus

INHIBITION OF FUNGAL GROWTH

This may be effected by the use of fungistatics or fungicides so that the outward growth of the keratin casts off infection, this has been accepted therapy for many years Further studies are required on the action of inhibitors on dermatophytes[2, 3]

MODIFICATION OF FUNGAL GROWTH

A knowledge of factors which determine in dermatophytes the change from parasitic to saprophytic growth and the reverse might make it possible to induce a change in behaviour of fungal growth on the human body A small group of cases of scalp infections due to *Microsporum audouini* was treated by keeping the affected areas covered with a medium similar to Sabouraud's agar at a high humidity, no beneficial effect was recorded This is an approach which requires greater knowledge of metabolism of ringworm organisms

TEMPORARY INHIBITION OF KERATIN FORMATION

X-rays or thallium, or depilating agents, have been used for this purpose which is practicable only for hair infection, though theoretically possible for nails Its efficacy in dealing with follicular infections is illustrated by the fact that radiological epilation, if complete, may be effective without the local use of fungicides Chloroprene and certain sterols bring about depilation but do not at present seem to have any value for therapeutic purposes

MODIFICATION OF KERATINS

The structure of keratins may be altered by physical or chemical means and such changes may result in inability of an infecting fungus to survive Fungi are also susceptible to alterations in humidity and probably also to changes in oxygen and carbon dioxide tension[4]

Keratin may be disorganized by the breakdown of cross-linkages

TREATMENT OF FUNGOUS INFECTIONS OF THE SKIN

THEORETICAL ASPECTS

A. J. E. BARLOW

The dermatophytes have three main sites of parasitic growth: (1) the horny layer; (2) the hair follicle in its lower two-thirds; and (3) the nail plate

In response to infection, including fungous infections, the human host can react in two main ways: (*a*) inflammation of varying degrees of acuteness, with the possible later development of [illegible]

curative and in such infections there is no problem of treatment. This type of reaction may also occur as the result of local treatment of anthropophilic infections, and in such instances the value of a fungicidal agent is frequently attributed incorrectly to its action on the infecting fungus rather than to its action on the skin structures It is probable that treatment has in some way disturbed the host-parasite balance so that the host has reacted to the infection with inflammation, a reaction by which it rids itself of the parasite Hypersensitive reactions characterize infections of the horny layer by *Trichophyton mentagrophytes* and certain other fungus species It is arguable how far such reactions are curative.

There is little doubt that treatment problems do not occur to the same extent in those fungous infections in which the host reacts vigorously as in those where little or no reaction results, for example, infections due to anthropophilic species Accuracy of diagnosis and careful assessment of cure are essential when handling these infections Diagnosis should always be confirmed not only by cultural identification but also by assessment of the extent of the infection microscopically, for *T rubrum* may be most ubiquitous and may occur in most unexpected sites Clinical impression of cure is insufficient; infected palms, soles and nails can look normal to the extent that even the patient may be convinced of cure yet the fungus may be shown to persist within them Cure should imply inability to identify the fungus in areas where previously it was present, and normality of appearance of tissues from a clinical point of view

with keratin The resultant compound may no longer be fungicidal and may also act as a barrier to further penetration.

In a thin sheet of soft keratin, as in the smooth skin, fungus may be superficial enough for its growth to be inhibited by a fungicide applied to the skin surface, but in the thicker horny layer of the sole and palm it is remote from effective fungicidal concentrations Similarly, the fungus may be vulnerable in the shallow follicle of a lanugo hair, but protected in the deeper follicle of a terminal hair

This has been in accord with our experience, not only with a variety of fungicides but also with cross-linking agents By virtue of the fact that the cross-linking agent, ninhydrin, combines with amino-acids to give coloured compounds, its degree of penetrability into keratin may easily be assessed This chemical has been used in *T. rubrum* infections of the palms and soles in the presence of surface agents and alcohol; after prolonged soaking the skin becomes purple By superficial paring with a scalpel, fungus can be demonstrated in the horny layer stained in the same way as the keratin In the deeper horny layer, however, the fungus is unstained The effect of ninhydrin on lesions of the thin keratin of the dorsum of the foot was to abate the infection as with conventional fungicides, but infection of the sole was unaffected

T rubrum infections can, in our experience, be cleared from smooth skin with Whitfield's ointment, aniline dyes and fungicides used on conventional lines It is doubtful, therefore, whether there is any wisdom in searching for new fungicides at this stage in our knowledge, and whether the assessment of fungicidal potency using *in vitro* culture methods is of real value In such methods the fungus is in its mycelial form, and different from its parasitic form, furthermore, the fungus is very vulnerable in cultures

For opening up keratin, lithium bromide, sodium metabisulphite, urea and thioglycolates have been used, and treatment has followed with cross-linking agents, including ninhydrin, dibromopropane and picric acid The inability to obtain adequate penetration of keratin has always proved an insuperable obstacle Keratolytics which produce more gradual degradation and which could be used over prolonged periods have also been used in the hopes that their action would overtake keratin production, give rise to a thinner horny layer and thereby allow better penetration of fungicides Salicyclic and benzoic acids were employed for this purpose in relatively high concentrations and were used in patients suffering from long-standing *T rubrum* infection of the palms and soles and of many nails The affected parts were soaked for half an hour daily in warm water and then painted with a solution of the two acids in spirit Very

between molecules by chemical agents (for example, keratolytic substances), or may possibly be made more "resistant" by the use of cross-linking agents[1]. The physical and chemical differences between soft and hard keratins are of importance in this respect in explaining the differences in behaviour of these keratins to both types of reagents.

Disulphide bonds, salt linkages, and hydrogen bonds are the main linkages between keratin molecules, and individual reagents may act predominantly on one particular linkage. Reducing agents such as sulphides, sodium metabisulphite and thioglycolates show their main action on disulphide bonds, whereas acids and alkalis act on salt linkages, and lithium bromide on hydrogen bonds. Keratolytics are used in the treatment of fungous infections to open up keratins so that fungistatic agents are more likely to reach sites of active fungal growth, but in so doing they probably increase the possibilities of fungal invasion It would, in theory, seem possible that keratins might be made impervious to fungal attack by the effect of cross-linking agents applied locally or by such agents administered systemically if excreted in keratins, sweat or sebum The influences of other organisms on the skin surface also require consideration, for it is possible that they may produce antibiotic fungicides, or alternatively, substances which themselves modify keratins

Most attention has been directed to the topical use of fungistatic substances and here the vexed question of penetration arises Penetration of keratin must be distinguished from percutaneous absorption implying the passage of substances through the skin into the systemic circulation Reagents which pass through the skin do not necessarily have the ability to diffuse into keratin Percutaneous absorption is most likely to be a property of substances which are fat-soluble and probably takes place through the sebaceous glands For therapeutic purposes it would appear necessary to obtain adequate concentrations of fungistatic agents in the deeper part of the soft keratin of the horny layer, in the lower two-thirds of the hair follicle, and in the nail plate Such penetration depends on many factors, not the least being the penetrability of soft and hard keratins themselves. Their structure is such that it is likely to be opened by water, alkalis and reducing agents, and permeation is most likely to be accomplished by molecules of small size These are accepted facts in the textile and tanning industries

It is necessary also to consider the effect of a chemical agent on keratin itself Many fungicides appear to act by combining with the proteins of a fungus and will probably, therefore, combine also

with keratin. The resultant compound may no longer be fungicidal and may also act as a barrier to further penetration.

In a thin sheet of soft keratin, as in the smooth skin, fungus may be superficial enough for its growth to be inhibited by a fungicide applied to the skin surface, but in the thicker horny layer of the sole and palm it is remote from effective fungicidal concentrations. Similarly, the fungus may be vulnerable in the shallow follicle of a lanugo hair, but protected in the deeper follicle of a terminal hair

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free exfoliation rapidly occurred and after a month of treatment no fungus could be found in either the palms or soles, although it was still present in the nails of these patients. Treatment was continued for a further month after which 15 nails were removed under general anaesthesia. The nail beds were then treated with ninhydrin and brilliant green, which in one instance produced a very acute inflammatory reaction. The nails grew again but, although of normal appearance, they were found to contain fungus one month later.

These cases have since relapsed with clinical evidence of nail infection although the palms and soles are still free from fungus. This experience appears to contradict the common belief that nail reinfection after removal is due to extension of infection from the adjacent skin, and that a prerequisite before nail avulsion is clearance of fungus from the surrounding skin areas.

At the present state of knowledge, there appears to be no place for surgical removal of nails infected with *T. rubrum*, with the possible exception of solitary nail involvement.

Various keratolytics applied to infected nails have also proved ineffective. There is unlikely to be any notable advance in treatment of intractable *T. rubrum* infections until more is understood of the fundamental factors controlling the parasitism of this fungus.

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TREATMENT OF FUNGOUS INFECTIONS OF THE SKIN

PRACTICAL ASPECTS

BRIAN RUSSELL

THE treatment of fungous diseases of the skin depends on the site of the infection and on the nature of the infecting organisms Treatment aims at both the control of the patient's infection and the prevention of its spread to others

RINGWORM OF THE SCALP

Infections due to Microsporum species.—When examination under [illegible] infection there is [illegible] by [illegible] ving [illegible] t of [illegible] ouini a history [illegible] animal source, [illegible] labrous lesions, [illegible] s, or rarely *M* [illegible] od's light of a *gypseum*, infection The exam[illegible] suspected animal (for example, a kitten or puppy) may reveal the source of infection, but veterinary euthanasia is unjustified without adequate evidence

Pending identification of the fungus it is useful to apply topical remedies The hair is cut very short over the whole head and the [illegible] thoroughly Whitfield's ointment, or some other [illegible] nail-brush, and [illegible] The parent is instructed to keep the child's head [illegible] ose-fitting linen skull-cap If the diagnosis of *M canis* infection is later established, this treatment can be continued for the 1-3 months during which this type of infection persists before undergoing spontaneous resolution

It is doubtful whether treatment with ointments has any value apart from minimizing aerial dissemination of fungous spores and mycelium in scales and hair fragments from the child's head, while the physician awaits the spontaneous shedding of the diseased hairs

In some cases, ointments seem to help by inducing an inflammatory reaction. Dithranol, phenyl mercuric nitrate, or other fungicides allegedly more powerful than Whitfield's ointment, do not appear to produce earlier resolution of infection.

If *M. audouini* is proved to be responsible it is rarely possible to succeed with topical remedies and recourse must be had to radiological epilation. Individual variations of response occur to radiotherapy and it may be that in a batch of 30 affected children one or more may fail to epilate completely, either at margins between the 5-inch diameter fields of 450r each, or in three converging lines where the wooden rods of the applicator have partially screened the rays. It is helpful to pare down these rods to minimize this screening effect. An exposure of 500r instead of 450r to each field also lessens the risk of incomplete irradiation in places without risk of scarring alopecia. (There can well be a +30 per cent or a −10 per cent variation in the radiation received at different sites.) Dr. Walter Shanks has suggested the replacement of the rods by two converging beams of light which when focused would enable distance to be judged accurately without interference with the passage of x-rays.

After treatment, the children continue to wear linen skull-caps and a fungicidal ointment is applied to the affected areas. During the phase of epilation the scalp is examined periodically with Wood's light. Preferably there should be no interference with the natural shedding of the individual hairs but occasionally it may be necessary to assist epilation by the gentle use of forceps or adhesive plaster. The absence of fluorescing hairs on 3 consecutive examinations at weekly intervals is the criterion of cure. A determined search must be made, using Wood's light, with the aid of the local authority, for other cases in the child's environment. Subclinical infections may be found. All infected cases should be treated concurrently as far as possible.

Cases which fail to epilate fully in part of the affected area are a serious problem unless puberty is close and spontaneous clearance therefore likely. Recourse may be had either to local irritant applications (for example dithranol) with a view to encouraging epilation, or to repeat radiological epilation of the affected area only (with due warning to the parents of the hazard involved). Thallium acetate is too toxic for serious consideration.

Other infections.—Anthropophilic endothrix types of infection due to *Trichophyton sulphureum*, *T. violaceum*, *T. tonsurans* and *T. schoenleini* are also treated by radiological epilation, there is no tendency to spontaneous resolution at puberty. All but the last of these 4 fungous infections are specially difficult to follow up since

Wood's light is of no avail, and cure can only be established by careful inspection with a hand lens and by microscopic examination of any suspected hairs that remain

Infections due to *T. mentagrophytes* and *T. verrucosum* (*T discoides*): These zoophilic infections present as kerion, and respond well to manual epilation and to the application of a fungicidal ointment. The temptation to incise a kerion should be resisted

RINGWORM OF THE BEARD

This infection, the true fig-like sycosis barbae, due to *T. mentagrophytes* or *T. verrucosum*, responds well to manual epilation accompanied by the topical application of fungicides If the least evidence of keloid formation develops, then a course of radiological treatment is indicated, such as 150r repeated weekly up to a total of 600r.

RINGWORM OF THE GLABROUS SKIN (tinea corporis, tinea circinata)

Treatment of this type of ringworm is not usually a difficult problem but examination must be made for coincident involvement of the scalp or nails. The application of magenta paint or of Whitfield's ointment is usually adequate and the lesion should be kept covered with a dressing to prevent inoculation of other sites Affected lanugo hairs should be manually epilated if they do not fall spontaneously

RINGWORM OF THE HANDS

[illegible]

from that of other glabrous sites and pustular folliculitis is dealt with by manual epilation *Trichophyton rubrum* infections are particularly resistant to all forms of treatment

RINGWORM OF THE AXILLAE AND GROINS

This condition is rare in females but relatively common in males It may be due to *Epidermophyton floccosum*, *T rubrum* or occasionally other Trichophyta Resolution, except in *T. rubrum* infections, may follow the application of magenta paint at night and the use of a 3 per cent salicylic acid dusting powder by day Concurrent treatment of other sites is essential

RINGWORM OF THE FEET

Enquiry must be made into the wearing of unsuitable footwear such as Wellington boots, shoes with crepe rubber soles, clogs at

work, coarse-knit woollen socks or shrunken socks. Orthopaedic disabilities such as pes planus or hallux valgus, also perniosis and hyperidrosis, may need attention The patient is instructed to wear white cotton in-socks which must be changed daily and boiled He should have his own bath-mat and he should scrub the bath immediately after use, to wash away infected epidermal debris. Magenta paint is applied twice a day to any active lesions, and 3 per cent salicyclic acid dusting powder or 25 per cent zinc peroxide powder is shaken from a canister between the toes and into the socks on rising These two remedies appear to be as effective as any proprietary fungicide. Secondary infection is treated by potassium permanganate (1 in 8,000) soaks, an appropriate antibiotic is sometimes indicated but often a 25 per cent zinc peroxide powder is more useful. In this way, infections with *E floccosum* and *T mentagrophytes* may be brought under control, but *T. rubrum* remains a law unto itself and no method of treatment is consistent in its efficacy

RINGWORM OF THE NAILS

There is no method available for curing ringworm of the nails Spontaneous resolution occasionally seems to occur, and extirpation of the nails may not be followed by recurrence in all of them.

PITYRIASIS VERSICOLOR

This infection due to a yeast-like organism affects the trunk more than the limbs Treatment necessitates investigation into hygienic practices, thus, there may be infrequent bathing and a habit of sleeping in a vest Hyperidrosis may be present and general causes for this symptom must be excluded

TREATMENT AND CONTROL OF CHILDHOOD RINGWORM

J M. BEARE

Scalp ringworm in the United Kingdom is usually due to one of 4 important fungi[16] Unfortunately, there is no known fungicide which when applied to scalp ringworm will shorten the duration of the disease other than, perhaps, by producing a chemical reaction which may cause the affected hair to fall out more quickly than it would do spontaneously. It is customary to rely upon symptomatic treatment but it is equally important to see that the source of infection is eliminated

The treatment of the infected child depends largely on the degree of inflammation associated with the infection, but the management of the outbreak, whether large or small, depends on the fungus responsible

In a case of scalp ringworm, a clinical diagnosis can usually be made by noting· (*a*) the degree of inflammatory response, (*b*) the size of the lesion in relationship to duration of infection, (*c*) the presence or absence of associated body lesions, (*d*) the presence or absence of fluorescence under Wood's light, (*e*) the history of possible contact with an infected animal (such as, cow, dog or cat), and (*f*) the name and address of the child and the school that the child attends[2]

COMMON VARIETIES OF SCALP RINGWORM

Microsporum audouini infections.—In the majority of these infections there is no reaction at all[3], only 2 per cent showing obvious inflammation At times of epidemics this fungus was found to be the most common cause of patchy dandruff in young children, the child usually having no other signs There is a considerable spontaneous cure rate and though infections will abate at puberty delay in treatment results in further spread of infection. In one outbreak of infection in a small town, no less than 27 per cent of the male school population were infected, and in some streets every child had [illegible]

must be almost unique It is therefore essential to render the child

free of infection at the earliest possible moment and this can be achieved only by radiological epilation Besides treating the child it is important to find the source of infection and evidence of spread of infection to other children. Every child in the home, the neighbourhood and the school should be examined with Wood's light; by such means we have eliminated an epidemic which involved over 1,000 children.

***Trichophyton sulphureum* infections.**—This is the main cause of human tinea capitis now that *M audouini* infection has abated, and it may well become troublesome in Great Britain during the next few years. It is already causing concern in America[4, 12], and in New Zealand[5] Whittle[19] has reported cases from the Cambridge area The condition is much less common than the animal varieties of ringworm and is usually symptomless though 28 per cent of cases are inflammatory[1] Tinea corporis is far more common in infections by this organism than in those due to *M. audouini*, and occasionally the nails are infected causing changes similar to and as intractable as those due to *Trichophyton rubrum* Scalp infections, whether inflammatory or non-inflammatory, often remit and radiological epilation should not be hurriedly instituted Furthermore, these cases are not so contagious as *M audouini* infections, and it is rare to get more than one child with scalp infection in a single household One can therefore temporize with a sticky ointment and a cover of the child's own hair over the infected area A certain proportion, however, will require radiological epilation eventually

The condition does not give fluorescence under Wood's light, so it is always difficult to find the source of infection, but the contacts in the home and school should be examined carefully since body ringworm due to this fungus is common

Microsporum canis.—Dog and cat ringworm is common all over the country and is much more common among cats than dogs It is doubtful whether there is much spread of infection from child to child In outbreaks investigated personally, all cases had direct contact with an infected animal It is, therefore, unnecessary to keep these children away from school[14] provided that the affected parts are covered (scalp lesions with greased normal hair) and that mixing at games is prevented Body lesions are more common than scalp lesions and the two may be found together in the same child Fifty per cent of all scalp infections are inflammatory[2] Non-inflammatory cases do not require radiological epilation and infections will clear within 6 months

It is important in *M canis* infections to discover the animal source of disease[8] and to have the animal destroyed because it cannot be

effectively treated. In one outbreak a seven-year-old tomcat was responsible for infecting his kittens and they in turn the children receiving them as pets[11] Adults handling the kittens were likewise infected as were dogs which came into contact with the kittens This particular tomcat was directly responsible for infecting 22 humans (including 8 adults), 13 kittens and 2 dogs. Local catteries are invariably responsible for large-scale outbreaks of *M. canis* infections[10]

Cats and dogs can easily be examined under Wood's light for fluorescent hairs, but considerable experience is required since small areas of infected fur behind ears or around the nose are difficult to find. Ringworm in dogs is much more easily seen clinically and diagnosis is seldom in doubt Cat and dog claws are often infected[9, 20].

***Trichophyton verrucosum (T. discoides)* infection.**—This is the common cause of cattle ringworm in Britain It invariably causes an inflammatory reaction requiring treatment by poultices and by removal of loose infected hairs, radiological epilation is never necessary. These cases are probably not infectious but, since spores from the fungus are known to remain alive for years in byres, fences and scratching posts[17], young cattle become infected year after year This is of little economic importance as healing is spontaneous within 4 months[13].

In the North of Ireland almost every farm worker gets ringworm at some time in his life. Beard infections are often the most severe and often leave permanent scars

Other fungi causing infections.—*Trichophyton mentagrophytes* is an uncommon infection, the animal source of which frequently remains in doubt (for example, cattle, mice) *Trichophyton equinum* occurs on gypsies' horses *Trichophyton schoenleini*, causing favus, *Trichophyton violaceum* and *Microsporum gypseum* are very uncommon causes of tinea capitis in the British Isles Whittle[18] described a small outbreak due to *M gypseum*, a fungus which may be a soil inhabitant[6, 7]

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TREATMENT OF VAGINAL MONILIASIS

R. F. JENNISON*

NYSTATIN AND GENTIAN VIOLET

PREGNANT and non-pregnant women with vaginal moniliasis from 5 out-patient units were referred to a special clinic for investigations and treatment by methods already described[3, 4]. The patients were divided into two comparable groups; each group was treated on 3 alternate days, one group having 2 pessaries containing 100,000 units of nystatin inserted, the other being painted with 1 per cent gentian violet. After treatment, 86 per cent of the 35 nystatin patients were free from *Candida* as compared with only 47 per cent of the 34 women treated with gentian violet, differences which are statistically significant The 5 failures with nystatin were all pregnant. Six patients were withdrawn from the gentian-violet series because of local reactions and, together with 12 gentian-violet failures, were treated with nystatin All 6 patients who reacted badly to gentian violet were cured by one course of nystatin There were 4 failures with nystatin out of 12 patients who had not been cured by gentian violet. Three of these received a second course of nystatin and 2 were cured

The results in all the 53 women who were treated with nystatin either primarily or secondarily are shown in Table 50 A notable feature of the nystatin treatment was the rapidity with which symptoms disappeared

The results of follow-up one month later showed that 21 per cent of the nystatin-treated patients had relapsed, compared with 46 per cent of those treated with gentian violet These patients had only short courses of nystatin and the results were regarded as extremely promising, especially when the ease of treatment with pessaries was compared with the previous system of painting with gentian violet

Sensitivity tests.—These were carried out by both blotting paper disc and serial tube dilution techniques on all organisms isolated (Fig 120) For routine use, discs containing 12 5 and 6 2 µg. of nystatin were selected In 90 strains tested at these strengths, the

* Acknowledgement for co-operation is made to the consultant staff of Saint Mary's Hospitals, Manchester, to the resident surgical officers, Dr J D Llywelyn-Jones, P A Manolis and R. M Thomson and to Mr P Stenton for assistance with the laboratory work.

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after 2 days' growth, suggesting that the early effect of nystatin at these concentrations was mainly fungistatic and not fungicidal. A concentration of 25 µg per millilitre of nystatin prevented *all* growth. The fungicidal powers were investigated further by estimating the rate at which *Candida* was killed when incubated in 50 µg. of nystatin per millilitre (Fig. 121) When the initial concentration of yeasts was of the order of 1 million per millilitre the viable count rapidly fell and all the organisms were dead at 24 hours. With initial concentrations

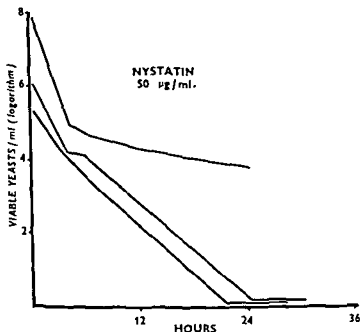


FIG 121 —Rate of decline of viable counts of yeasts in presence of 50 µg per millilitre of nystatin (*By courtesy of the Department of Medical Illustration, Royal Infirmary, Manchester*)

of 100 million yeast cells per millilitre the organisms were not killed in 24 hours, though they were considerably reduced in number

The relationship between concentration of the antibiotic and number of organisms was of great importance in these *in vitro* studies, and the rapid relief of symptoms in patients treated with nystatin suggested that concentrations of the appropriate proportions were in fact being obtained in the vagina

TABLE 50

RESULTS OF TREATMENT OF VAGINAL MONILIASIS WITH NYSTATIN IN PREGNANT AND NON-PREGNANT WOMEN

	Patients	*Cured*	
		Number	*Per cent*
Pregnant	22	18	82
Non-pregnant	31	29	93
Total	53	47	88

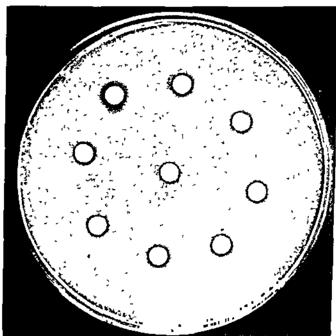


Fig. 120 Zones of inhibition around discs containing from [illegible]

zones of inhibition were 10–22 millimetres All strains of *Candida* isolated at this time and tested by this method were found to be sensitive The average minimal inhibitory concentration of nystatin was 3 2 µg per millilitre after 1 day and 12·5 µg per millilitre

AMPHOTERICIN B

Recently another anti-fungal antibiotic (amphotericin B) has been available and a small trial has been conducted In all, 54 women were treated with vaginal tablets containing 25 milligrams or 50 milligrams of amphotericin B One tablet was inserted each night for 15 nights Nineteen women were treated with 25 milligram tablets, but 2 did not attend after completion of treatment Four of the remainder were pregnant and the treatment had no effect Of the other 13, 11 were free from yeasts at the end of treatment but 2 of these had recurred 1 week later Thirty-five women were treated with 50 milligram tablets, 4 of whom did not attend after treatment Seven were pregnant and only 1 was cleared; 21 of the other 24 were free from yeasts at the end of treatment but 5 recurred within 2 weeks

The overall cure rate estimated 1-2 weeks after the cessation of treatment was 53 and 54 per cent respectively, with the 25 milligram and 50 milligram tablets The amphotericin tablets seemed, therefore, to be very much less effective in pregnancy than nystatin and slightly less effective in non-pregnant women The recurrence rate appeared to be higher than that following nystatin therapy

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TREATMENT SUMMARY

The results of treatment of all patients treated with nystatin or gentian violet and adequately followed up in the years 1956–57 are summarized in Table 51. The recent improvement in results is probably explained by the longer course of treatment latterly employed. Since the end of 1955, there have been 91 per cent of cures out of 172 pregnant and non-pregnant women with vaginal moniliasis treated with nystatin as compared with 57 per cent of cures in 71 women treated with gentian violet Oral tablets were combined with pessaries in 9 patients, but there was no evidence that the addition of such oral therapy was of any value

TABLE 51

RESULTS OF TREATMENT AND FOLLOW-UP IN PATIENTS WITH VAGINAL MONILIASIS (1956-57)

	Number treated	*Number cured*	*Per cent cured*	*Per cent cured in earlier trial (1955-56)*
Nystatin	119	78 (+36)*	96	86
Gentian violet	35	17 (+ 6)*	66	47

*Clinically cured only

The majority of the courses of nystatin treatment have consisted of 1 pessary nightly for 15 nights Although most patients were cured by this 2-week course, a number, especially among the pregnant women, required longer or repeated courses The length of the course of treatment also seems to have an effect on the relapse rate Using 1 pessary nightly for 1 week, Barr[1] treated 64 women of whom 10 became reinfected later, giving a relapse rate of at least 15 per cent Using a similar dosage, Browne[2] treated 25 women, with a relapse rate of 20 per cent Pace and Schantz[5] treated 38 women with a similar course, with 29 per cent relapses, whereas in 2 smaller groups treated with 2 pessaries nightly for 1 week and 1 pessary nightly for 2 weeks, less than 15 per cent relapsed

In our first series when only 2 pessaries on 3 occasions were used, the relapse rate 1 month after treatment was 21 per cent, but when the 2-week course was instituted, only 5 patients required a second course and 5 recurred later out of 113 treated, a relapse rate of less than 9 per cent

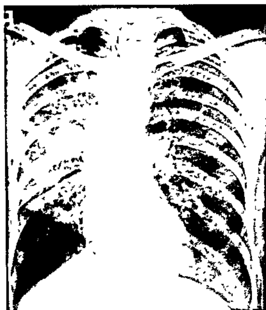


FIG. 122 —Chest radiograph of patient with actinomycosis of the right lung erroneously diagnosed as carcinoma and treated by pneumonectomy (*By courtesy of the Editors of Thorax*)

of sulphonamides, the prognosis improved considerably Some patients, however, failed to respond to one sulpha drug and yet responded well to another, particularly to sulphadiazine There were cases in which no effective form of sulphonamide was found There are many cases in the literature reported as cured based on a follow-up period of only a few months Such cures are not acceptable since actinomycosis is often a disease comparable in its chronicity to tuberculosis. Results of cure should be based on a follow-up over some years

Penicillin.—This has made it possible to cure almost all cases of actinomycosis provided high enough doses are given for a sufficiently long period. Only 2 cases encountered failed to respond clinically to penicillin. In one of these Garrod found that the culture of *A israeli* grown was initially fully sensitive *in vitro* to penicillin but its sensitivity decreased tenfold during treatment, in the other the actinomycete had cultural characteristics which were very atypical of *A. israeli* *In vitro* penicillin-sensitivity tests are not always reliable For example, one case in which the organism initially grown was reported elsewhere as being highly penicillin-resistant *in vitro*

TREATMENT OF ACTINOMYCOSIS

O. S TUBBS

ACTINOMYCOSIS is the one form of fungous disease which responds well to treatment. The experience recorded below deals with 23 patients seen at the Thoracic Unit of St. Bartholomew's Hospital, London, and in which the disease was predominantly in the thorax They are included in a review of the subject describing the results of treating 85 cases by Bates and Cruickshank[1].

DIAGNOSIS AND TREATMENT

The successful treatment of actinomycosis depends almost entirely upon making the correct diagnosis *It should be emphasized that* many cases of infection of the chest by *Actinomyces israeli* do not present with the classical findings of multiple discharging sinuses, the occurrence of which makes diagnosis easy. The cases in which the diagnosis is most likely to be missed are those with a chronic pulmonary lesion producing a radiological shadow which may be ascribed to carcinoma or to some chronic infection other than actinomycosis As a result the patient may be submitted unnecessarily to resection of a lobe or even a whole lung (Fig 122). This is brought out clearly in the paper by Bates and Cruickshank for 7 of the 85 cases underwent resection of a lobe or lung and in none of these 7 cases was a correct pre-operative diagnosis made Prior to the introduction of the sulphonamides, treatment aimed at improving the general condition of the patient, and increasing resistance to the disease by vaccines Massive doses of iodides were given by mouth presumably to aid absorption of fibrous tissue Oxidizing agents, such as zinc peroxide, were applied locally to destroy anaerobic conditions favouring the growth of *A israeli* Radiotherapy was sometimes used with a view to increasing local resistance to the disease Surgery was employed primarily to drain empyemas and abscesses and to excise diseased portions of the chest wall. Successful results from surgery occurred almost entirely in cases with localized empyema (Figs 123 and 124)

Before the sulphonamides and antibiotics became available, the results of treatment were very bad indeed, Bates and Cruickshank found that during this era, out of 29 patients whom they studied, only 4 are known to have recovered Following the introduction

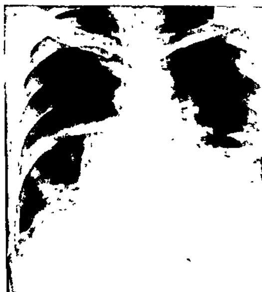


FIG 125 —Chest radiograph showing bilateral loculated empyema due to actinomycosis (*By courtesy of the Editors of Thorax*)



FIG. 126 —Chest radiograph of same patient as Fig. 125 following bronchopneumonic spread of the infection. (*By courtesy of the Editors of Thorax*)



Fig. 123.—Chest radiograph of male aged 53 years with large unilocular right-sided actinomycotic empyema (*By courtesy of the Editors of Thorax*)



Fig 124—Chest radiograph of same patient as Fig 123 after drainage of the empyema

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FIG. 128—Longstanding actinomycosis of the chest wall involving the sternum (By courtesy of the Editors of *Thorax*)

Other antibiotics.—For the very exceptional case which proves resistant to penicillin alone, either an additional drug known to be effective in combination with penicillin should be administered (for example, streptomycin) or, alternatively, the penicillin may be omitted and some other antibiotic drug given, preferably chosen on the basis of *in vitro* sensitivity test results One patient whose organism proved resistant to penicillin *in vitro* and who also failed to respond clinically to penicillin was given a long course of terramycin. This led to marked improvement in an extremely chronic suppuration of the chest wall of 15 years' duration (Fig 128). Terramycin apparently eradicated the *A israeli*, but death occurred later from secondary haemorrhage from an internal mammary artery, the patient having repeatedly refused resection of the osteomyelitic sternum.

CONCLUSIONS

In general, surgery rarely has any part to play in the treatment of thoracic ... the ... ially ... ions

responded to penicillin therapy in dramatic fashion. (A subculture of the organism was subsequently examined by Garrod and found to be as sensitive as the Oxford staphylococcus) Penicillin therapy should, therefore, be proceeded with as soon as *A. israeli* is isolated regardless of the results of sensitivity tests

Penicillin should be given in sufficient amount and continued for long enough to make as sure as possible that the patient is cured on a single course of treatment. From an analysis of those cases which relapsed it may be concluded that 2,000,000 units given 8-hourly for 6 weeks followed by 600,000 units of distaquaine penicillin given daily for another 6 weeks are necessary to make the



Fig. 127 —Chest radiograph of same patient as Figs. 125 and 126 following final course of penicillin (*By courtesy of the Editors of Thorax*)

chance of relapse remote The following case is described as an example of relapse occurring due to inadequate penicillin therapy:

A man aged 42 years first came to hospital with an empyema on the right side from which micro-aerophilic streptococci were grown He was given 13 days' treatment with 400,000 units of penicillin daily for this streptococcal infection He subsequently returned with a subcutaneous abscess in the right axilla and with an empyema on the other side (Fig 125) both of which were drained Loculation of the empye- [illegible] on both sides and it was then

of pus in the pleural cavity should be drained by rib-resection. Chronic pulmonary infection usually clears up with penicillin therapy alone but exceptionally the disease may cause much bronchiectasis and fibrosis and this may call for resection if symptoms arise as the result of secondary infection. In this case the operation is done, *not* for actinomycosis, but for structural damage caused by the infection.

REFERENCE

[1] Bates, M., and Cruickshank, G (1957). "Thoracic Actinomycosis" *Thorax*, 12, 99

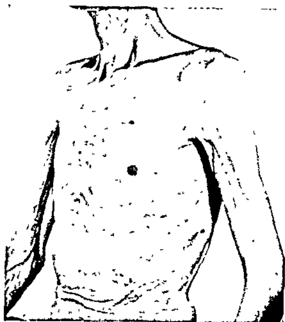


FIG 129—Localized abscess in left pectoral region due to actinomycosis (*By courtesy of the Editors of Thorax*)

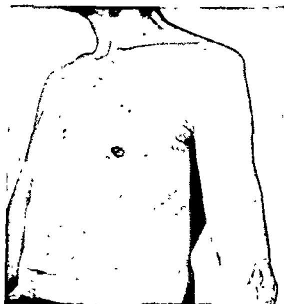
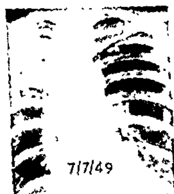
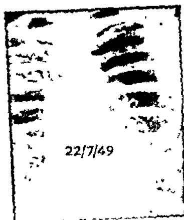


FIG 130—Same patient as shown in Fig 129, 5 months later following penicillin treatment (*By courtesy of the Editors of Thorax*)

This attack subsided but was followed by another (Fig. 133) affecting the basal zone on the right side and also giving rise to left lower lobe collapse which persisted for a year. Subsequently, more shadows were seen in other zones (Figs 134 and 135) and the left lower lobe



(a)



(b)



(c)

Fig 132 a, b, c—Resolution of right upper lobe consolidation in 2 weeks, July 1949

began to re-expand and finally appeared normal Bronchograms showed saccular bronchiectasis of the right upper lobe, the original site of the pneumonia At bronchoscopy, a rounded tumour-like mass was seen to be occupying the left lower lobe bronchus In

TREATMENT OF ASPERGILLOSIS

N. S PLUMMER

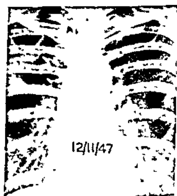
BRONCHO-PULMONARY ASPERGILLOSIS may be divided into 3 main types: (*a*) allergic, (*b*) saprophytic, including aspergillus mycetoma, (*c*) septicaemic or pyaemic.

THE ALLERGIC TYPE

It should perhaps be stated that when the allergic type of aspergillosis was described in 1952[1] no reference had been seen to this condition in the literature. Later, however, such a reference was found in the *Manual of Clinical Mycology* by Conant and his colleagues[2] in which it was stated briefly that sensitization had occurred to the



(a)



(b)

FIG 131 a, b —Resolution of right upper lobe consolidation in 3 weeks, 1947

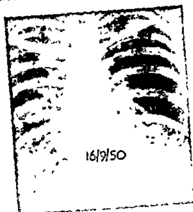
aspergillus, giving rise to prolonged illness in which transient lung shadows were seen by radiography The main features of the condition are recurrent attacks of pyrexia, typical allergic sputum with plugs, the finding of *Aspergillus fumigatus* in the sputum, blood eosinophilia, and changing serial radiograph shadows

Case history.—The first patient with this condition seen by the author, in 1947, had a pneumonic episode which resolved in about 3 weeks (Fig 131) In 1949 the disease recurred (Fig 132) with fever, expectoration of copious sputum, but no asthmatic symptoms

administration appeared to affect the course of disease and the sputum remained positive for *A. fumigatus*. The patient became ill intermittently with similar episodes over a period of 4 years For the last 4 years, however, fungus has not been detected in her sputum



(a)



(b)



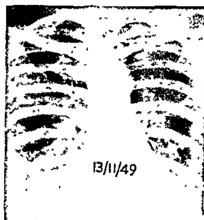
(c)

FIG 134 a, b, c —September 1950, the lower lobe is beginning to re-expand

in spite of many culture examinations Evidence of sensitization as indicated by blood eosinophilia and transient lung shadows has vanished completely and the patient is now essentially a case of bronchiectasis with minor exacerbations of bronchitis

Presence of pneumonia and bronchiectasis.—It is important to note,

each episode the patient responded well to penicillin, by the fourth day of therapy the temperature fell, the sputum became more mucoid and showed fewer brownish-yellow plugs containing aspergillus The plugs did not, however, disappear entirely from the

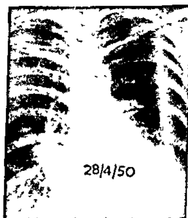


(a)



(b)

FIG 133—(a) Consolidation in right lower lobe and collapse of left lower lobe, November 1949 (b, c) Also middle lobe consolidation and collapse of left lower lobe, April 1950



(c)

sputum by the time the next pyrexial episode occurred and blood eosinophilia persisted 4 4′-diamidinophenylamine hydrochloride was given by inhalation, but detectable blood levels of the drug were not recorded The same drug was administered intravenously until a total of 1 2 grammes had been given Neither route of

administration appeared to affect the course of disease and the sputum remained positive for *A. fumigatus* The patient became ill intermittently with similar episodes over a period of 4 years. For the last 4 years, however, fungus has not been detected in her sputum



(a)



(b)



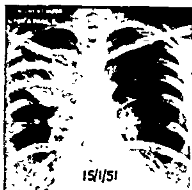
(c)

Fig 134 a, b, c —September 1950, the lower lobe is beginning to re-expand

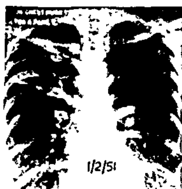
in spite of many culture examinations *Evidence of sensitization as indicated by blood eosinophilia and transient lung shadows has vanished completely and the patient is now essentially a case of* bronchiectasis with minor exacerbations of bronchitis

Presence of pneumonia and bronchiectasis.—It is important to note,

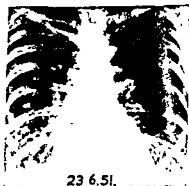
therefore, that the allergic type of aspergillosis may ultimately resolve spontaneously. Treatment with penicillin has been used in another case with similar good effect, presumably because of the superadded bacterial pneumonia almost invariably present. Antibacterial antibiotics should not be withheld on account of the presence of fungus in the sputum. In another case cortisone and hydroxystilbamidine



(a)



(b)



(c)

FIG 135—(a, b) Recurrence in right upper lobe with resolution in 2 weeks, January 1951 (c) June 1951, the left lower lobe has almost fully re-expanded

(2-hydroxy-4 4'-diamidinostilbene) in combination were administered A total dosage of 2½ grammes of hydroxystilbamidine was given over about 3 weeks but it had, apparently, no effect in hastening the recovery of a pneumonic episode

Sometimes after years of illness with recurring symptoms, radiological shadows and fungus-positive sputum, patients recover The eosinophilia disappears and chest radiographs become normal yet

A fumigatus continues to appear in sputum samples Treatment by surgery may be contemplated in view of the fact that in all the author's cases there has been underlying bronchiectasis In 3 such cases removal of a lobe infected by aspergillus was followed by the development of recurring lung shadows in the other lung and the sputum remained fungus-positive.

THE SAPROPHYTIC TYPE

The saprophytic type of aspergillosis comprises a heterogeneous group of conditions in which *A. fumigatus* secondarily invades lungs affected by pulmonary tuberculosis, sarcoidosis, bronchiectatic cysts, abscesses, bronchial carcinoma, pneumonia and other processes In such cases, it is doubtful whether specific treatment of the aspergillosis is necessary since treatment of the primary lung disease will probably lead to recovery from the fungous infection In cases of pneumonia where there is free invasion of the lung by the aspergillus it is probable that the fungous infection is superimposed upon bacterial infection due, for example, to staphylococcus Though it would seem reasonable to treat such patients with an antifungal drug no benefit was apparent in 4 cases treated with hydroxystilbamidine. In such cases, treatment with an antibacterial antibiotic has usually been effective, although *A fumigatus* has persisted [illegible]

[illegible]

bronchial epithelium; in 2 cases where this was absent granulation tissue surrounded the mass. In some instances a mycetoma has given rise to no symptoms during a 10-year period prior to resection Recurrent haemoptysis frequently occurs, however, and this is the usual way the patient presents for treatment Mycetomas can usually be resected fairly readily though complications may follow such a procedure (for example, bronchial fistula leading to empyema). Experience has led to the conclusion that aspergillus mycetomas should not be treated by surgery unless there is evidence that such enlargement is taking place as to render them dangerous to the health of the patient, or unless exact diagnosis is in doubt It is conceivable that their presence could produce a condition of hypersensitivity to aspergillus of the kind described above, but this occurred in 3 patients after resection In 1 case it was found that a typical mycetoma was sited within a necrotic carcinoma

Treatment of empyema infected secondarily with *A fumigatus* is very difficult On theoretical grounds one should instil an antifungal

drug into the empyema cavity, but in 2 or 3 cases so treated this was *not effective in removing the fungus.*

The septicaemic type of aspergillosis has not been encountered

CONCLUSIONS

It is, perhaps, not surprising that the parenteral administration of antifungal drugs is ineffective in broncho-pulmonary aspergillosis since, in the majority of cases, the fungus lies beyond the reach of the blood stream—for example, in the lumen of a bronchus or cyst. Furthermore, in cases of local or systemic invasion by fungus there is usually, if not always, some underlying condition such as pneumonia or abscess formation or some debilitating disease like carcinoma or agranulocytosis to be reckoned with. Administration of iodides has been tried but without any apparent effect on the course of the disease

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[1] Hinson, K F. W, Moon, A J., and Plummer, N S (1952) "Broncho-pulmonary Aspergillosis" *Thorax*, 7, 317

[2] Conant, N F., Smith, D T, Baker, R D, Callaway, J L, and Martin, D S (1944) *Manual of Clinical Mycology*. 1st ed Philadelphia and London, Saunders

EXPERIENCES WITH THE THERAPY OF 60 CASES OF DEEP MYCOTIC INFECTIONS

M. L. FURCOLOW*

WITH the exception of actinomycosis and nocardiosis the commonly employed antimicrobial agents have proved to be ineffective in deep mycotic infections Recently we have used a new antibiotic, amphotericin B, in the treatment of histoplasmosis and cryptococcosis with promising preliminary results

Criteria for the diagnosis of histoplasmosis, are described in detail elsewhere[9].

REVIEW OF ANTECEDENT THERAPEUTIC TRIALS

AROMATIC DIAMINES

Following a report by Elson[8] in 1945, a series of reports[4, 5, 22, 27] appeared in the literature indicating favourable results obtained with stilbamidine and later 2-hydroxystilbamidine in the treatment of blastomycosis Of the 5 cases of systemic blastomycosis treated by our group results have likewise been favourable All of our cases received 2-hydroxy-4:4′-stilbenedicarboxamide (2-hydroxystilbamidine, Merrell) intravenously in a daily dose of 225 milligrams In 3 cases complete resolution occurred after a 30-day course of therapy The remaining 2 patients required a second course before complete recovery

In view of the remissions produced in blastomycosis, 2 cases of chronic progressive cavitary histoplasmosis and one with disseminated disease were treated with a similar therapeutic regime without demonstrable effect *In vitro* studies[23] indicated that the body fluid levels required to inhibit the growth of *Histoplasma capsulatum* far exceed those which can be attained

With the development and availability of the analogue, 2-amino 4 4′-stilbenedicarboximide (Aminostilbamidine, Merrell), 3 patients with cavitary histoplasmosis were treated with 250 milligrams intravenously daily for 30–60 days No definite therapeutic effect could be demonstrated

MRD-112.—In 1949, Tilford and his colleagues[25] described the

* In collaboration with P H Lehan, J Lewis Yates, C A Brasher and H W Larsh

Acknowledgement ... "owing physicians who co- ... homa and Texas: Doctors M Fitzpatrick, Marion J Rogers and R Weber

properties of β-diethylaminoethyl fencolate (MRD-112, Merrell) Since then various reports on the "beneficial effect" of MRD-112 on the course of cavitary histoplasmosis have appeared[12, 19, 20, 21]. We have undertaken clinical trials with this agent in a total of 14 patients; 2 with cryptococcal meningitis, 2 disseminated histoplasmosis and 10 cavitary histoplasmosis. All received daily intravenous doses from 150 to 250 milligrams for periods of 30 to 60 days The administration of MRD-112 did not alter the course or fatal outcome in either cryptococcal meningitis or disseminated histoplasmosis Similarly, no effect was demonstrated among those with cavitary histoplasmosis.

Ethyl vanillate.—Christie and his colleagues[3] in 1951 reported favourable results with ethyl vanillate in the treatment of disseminated histoplasmosis. Since that time a number of favourable results and failures have been reported in the literature[7, 17, 28]. Although we have had no experience with this drug, the apparently erratic results obtained by others and the narrow therapeutic index led us to search for a better therapeutic agent.

Nystatin.—In 1951 Hazen and Brown[11] described an antifungal agent derived from several species of *Streptomyces*. This antibiotic was first called "fungicidin" but subsequently renamed nystatin (mycostatin, Squibb) Animal studies by Campbell[2] revealed that nystatin was an effective *in vivo* antifungal agent Negligible absorption from the gastro-intestinal tract precluded the use of oral nystatin in the treatment of the deep mycotic infections. Trials with an intravenous preparation were undertaken in 4 patients with cavitary histoplasmosis and 1 with cavitary coccidioidomycosis In 4 of these marked side reactions consisting of chills, fever, nausea and vomiting prevented a full course of therapy One case with cavitary histoplasmosis received 200,000 units daily for 62 days without demonstrable therapeutic or toxic effects

Amebacide CT 686.—This compound [1, 2-Bis-para (n-hexylmethylaminomethyl) phenoxy ethane dihydrochloride] showed considerable activity *in vitro*[13] against a variety of fungi Since it was relatively soluble in water, therapeutic trials with an oral preparation were undertaken Fourteen cases of cavitary histoplasmosis received oral doses ranging from 48 to 300 milligrams daily for a [illegible]

THERAPEUTIC TRIALS WITH AMPHOTERICIN B

Members of the Squibb Institute of Medical Research and their

associates have recently isolated and described a new antifungal antibiotic derived from a soil *Streptomyces*[2, 10, 11, 26]. Studies by this group have indicated that amphotericin B is a weak base and is amphoteric as demonstrated by its greater solubility in acidic or basic aqueous alcoholic solvents. While the exact chemical structure of this compound is not known, its molecular formula has been established as $C_{46}H_{73}NO_{20}$[6]. Ultra-violet absorption maxima indicate that there is a conjugated hexaenic or heptaenic system in amphotericin B

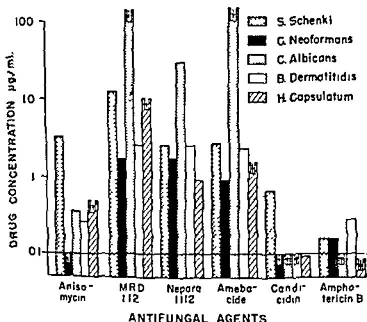


FIG 136—Comparison of the concentrations of 6 antifungal agents necessary to produce 50 per cent inhibition of the yeast phase of pathogenic fungi recently isolated from clinical cases

IN VITRO STUDIES

In vitro studies by Gold and his colleagues[10] have revealed that amphotericin B does not have antibacterial activity but is effective in inhibiting the growth of the yeast and yeastlike fungi. Recent *in vitro* studies[14] in this laboratory with 5 strains of yeast phase *Histoplasma capsulatum* have shown that less than 0.1μg per millilitre of amphotericin B was needed to produce 50 per cent inhibition of all strains. Fig 136 compares the relative activity of amphotericin B

TABLE 52

CRYPTOCOCCAL MENINGITIS. SUMMARY OF CLINICAL AND LABORATORY DATA ON CASES TREATED WITH AMPHOTERICIN B

Pre-treatment		Therapeutic regime		Post-treatment		Comments
Case number and clinical condition	*Cultures*	*Route, dose and frequency*	*Number of doses*	*Clinical response*	*Cultures*	
1 (56 W M) Moribund	Spinal fluid + Gastric + Urine +	Oral 2 gm /q d	4	Expired	Positive	Post mortem denied
2 (50 W F) Good	Spinal fluid +	Oral 2 gm /q d	30	Good	Negative	Improving prior to therapy Asymptomatic 1 year
3 (63 W M) Moribund	Spinal fluid +	Intravenous 50 mg /q d	7	Expired	Positive	Diabetic—5 years Post-mortem culture positive
4 (37 W M) Poor	Spinal fluid + Skin +	Intravenous 50 mg /q d 80 mg /q d 100 mg /q o d	4 9 4	Good	Negative Negative	Concomitant lymphoma. Skin lesions healed. Asymptomatic—5 months
5 (62 W M) Poor	Spinal fluid +	Intravenous 100 mg /q d 50 mg /q d 50 mg /q o d	8 4 10	Fair	Negative	Well—5 months CSF protein remains elevated
6 (31 W F) Fair	Spinal fluid +	Intravenous 50 mg /q d 100 mg /q o d	13 27	Good	Negative	Asymptomatic—4 months Spinal fluid normal

and Louria[18] have shown that in mice amphotericin B is an effective in vivo antifungal agent with a high therapeutic index.

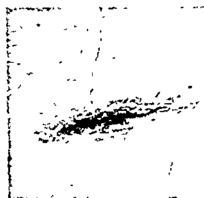


Fig 137.—(Case 4 in Table 52.) Chronic cutaneous lesion overlying the 11th rib posteriorly as it appeared the day therapy was instituted Present without change for 3 months *C neoformans* recovered from lesion

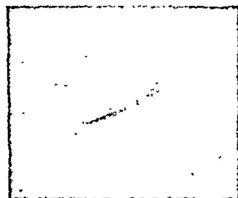


Fig 138.—Same lesion as it appeared approximately 2 weeks after instituting intravenous amphotericin B therapy No evidence of recurrence after 4 months

Cryptococcosis.—All the cases in this series presented clinical evidence of meningitis and *Cryptococcus neoformans* was readily recovered from the spinal fluid In each instance pathogenicity was confirmed by intracerebral mouse inoculation Table 52 outlines the pre-treatment status and cultures, the therapeutic regime and response of these patients. It should be noted that 3 out of 6 patients recovered, 1 improved and 2 (admitted *in extremis*) died

TABLE 53

DISSEMINATED HISTOPLASMOSIS. SUMMARY OF CLINICAL AND LABORATORY DATA ON CASES TREATED WITH AMPHOTERICIN B

Pre-treatment		Therapeutic regime		Post-treatment		
Case number and clinical condition	*Cultures*	*Route, dose and frequency*	*Number of days treated*	*Clinical response*	*Cultures*	*Comments*
7 (4 W.F.) Fair	Bone marrow −	Oral 1 gm/q d	90	Temporary improvement	Negative	Relapse after discontinuation of therapy
	Lymph node +	2 4 gm/q d	120	Questionable	Negative	Has persistent hepatosplenomegaly
8. (50 W M) Poor	Bone marrow + Sputum + Laryngeal lesion +	Oral 2 gm/q d 4 gm/q d	60 78	Temporary improvement Expired	Negative Not done	1 week after discontinuing therapy cultures again positive Received cortisone for adrenal insufficiency. Suddenly expired 3 months after therapy
9 (5mo C F) Moribund	Bone marrow +	Intravenous 5 mg/q d	3	Expired		Post-mortem cultures positive
10 (69 W M) Moribund	Sputum +	Intravenous 50 mg/q d	2	Expired		Post mortem denied
11 (69 W M) Moribund	Sputum + Bone marrow +	Intravenous 100 mg/q d 50 mg/q d 50 mg/q o d, oral 2 gm/q d	6 15 34	Good	Negative	Asymptomatic 6 months Received oral therapy 2 additional months
12 (56 W M) Poor	Oral ulcer +	Intravenous 50 mg/q d 100 mg/q o d	23 17	Good	Negative	Complete healing oral lesion. 20 lb weight gain Asymptomatic 4 months

Despite repeated cultures, in no instance was the organism recovered after 2 weeks or more of intravenous therapy. One case had, in addition to meningitis, several cutaneous lesions of 3 months' duration due to *C. neoformans* Figs 137 and 138 present the rather dramatic healing of one of these lesions while under therapy. In all but 1 case the cerebrospinal fluid cell count, sugar and protein reverted to normal

Histoplasmosis.—As a result of our interest in this disease and our geographical location suitable cases were readily available All cases selected for therapeutic trial were proved by isolation of the fungus by culture. Division of cases into the various classifications is based on criteria previously outlined by Furcolow[9].

Progressive disseminated histoplasmosis—Six cases of this almost universally fatal entity received amphotericin B (Table 53) The first 2 cases received only oral drug and in both instances temporary clinical improvement and negative cultures were obtained while under active therapy Both patients relapsed approximately 1 month after the therapy was stopped The remaining 4 cases received intravenous therapy. While 2 of these patients expired, only 3 doses were administered before their demise The remaining cases demonstrated rather dramatic clinical improvement and cultural conversion. One case had a granulomatous ulcerating lesion on the floor of the mouth shown to be due to *H capsulatum* Figs 139 and 140 depict this lesion prior to and 3 weeks after therapy was instituted

Chronic progressive pulmonary histoplasmosis—Six patients who were classified in this category were selected for therapeutic trials with oral and intravenous amphotericin B Of these, 5 had cavitary diseases and the remaining case had long-standing apical disease without cavitation Only cases in which *H capsulatum* had been repeatedly isolated from the sputum were selected for study In an effort to minimize the possible effect of bed-rest and general supportive measures all cases were observed for 2–3 months before therapy was instituted Only 1 patient (Case 17) had significant clinical and radiographic improvement on bed-rest alone Drug therapy was withheld in this case until his clinical status and radiographs were stable

The problems of evaluation of therapy on a short-term basis for this type of disease are comparable to those with advanced pulmonary tuberculosis The criteria used in evaluating response to therapy were (*a*) changes in clinical status, (*b*) radiograph changes, (*c*) complement fixation titre changes, (*d*) conversion of sputum cultures A summary of the clinical and laboratory data on these

TABLE 53

DISSEMINATED HISTOPLASMOSIS. SUMMARY OF CLINICAL AND LABORATORY DATA ON CASES TREATED WITH AMPHOTERICIN B

Pre-treatment		Therapeutic regime		Post-treatment		
Case number and clinical condition	*Cultures*	*Route, dose and frequency*	*Number of days treated*	*Clinical response*	*Cultures*	*Comments*
7. (4 W F) Fair	Bone marrow –	Oral 1 gm /q d	90	Temporary improvement	Negative	Relapse after discontinuation of therapy
	Lymph node +	2 4 gm /q d	120	Questionable	Negative	Has persistent hepatosplenomegaly
8 (50 W M) Poor	Bone marrow + Sputum + Laryngeal lesion +	Oral 2 gm /q d 4 gm /q d	60 78	Temporary improvement Expired	Negative Not done	1 week after discontinuing therapy cultures again positive Received cortisone for adrenal insufficiency Suddenly expired 3 months after therapy
9 (5mo C F) Moribund	Bone marrow +	Intravenous 5 mg /q d	3	Expired		Post-mortem cultures positive
10 (69 W M) Moribund	Sputum +	Intravenous 50 mg /q d	2	Expired		Post mortem denied
11 (69 W M) Moribund	Sputum + Bone marrow +	Intravenous 100 mg /q d 50 mg /q d 50 mg /q o d oral 2 gm /q d	6 15 34	Good	Negative	Asymptomatic 6 months. Received oral therapy 2 additional months
12. (56 W M) Poor	Oral ulcer +	Intravenous 50 mg /q d 100 mg /q o d	23 17	Good	Negative	Complete healing oral lesion 20 lb weight gain Asymptomatic 4 months

CHRONIC PROGRESSIVE PULMONARY HISTOPLASMOSIS. SUMMARY OF CLINICAL AND LABORATORY DATA ON CASES TREATED WITH AMPHOTERICIN B

Pre-treatment					*Regime*		*Post-treatment*					*Comments*
Case number and condition	*Radiograph*	*C F titre*	*Sputum*		*Route, dose* and frequency*	*Number of doses*	*Clinical response*	*Radiograph improvement*	*C F titre*	*Sputum*		
			Amount	*Culture*						*Amount*	*Culture*	
13. (42 W M) Fair	Far advanced cavitation bilaterally	1 32	30–60 ml	+	Oral 4-6 gm	330	Good	Slight to moderate	1.16	5–10 ml	–	Cavity (1 5 cm) right sub apex disappeared Cultures negative for 9 months
14 (64 W M) Poor	Far advanced cavitation bilaterally	1 256	30–90 ml	+	Oral 4-8 gm q d Intravenous 100 mg q d	240 9	Fair	Moderate following intravenous	1 132	15–30 ml	+	Improved remarkably with intravenous drug, but received tetracycline at same time
15 (37 W M) Fair	Moderately advanced cavitation bilaterally	1 8	50–60 ml	+	Oral 4 mg q d Intravenous 50 mg T I W	50 31	Good	Marked	1.8	5–10 ml	–	Febrile response following intravenous injection daily Under therapy 4 months
16 (38 W M) Fair	Far advanced cavitation bilaterally	1 8	30–60 ml	+	Oral 4 gm q d Intravenous 66 mg T I W	50 31	Mod Good	Moderate		3–5 ml	–	Under therapy 4 months. 30 lb weight gain
17 (35 W M) Fair	Moderately advanced non-cavity apical disease	1 64	15–20 ml	+	Oral 4–6 gm q d Intravenous 50 mg T I W	55 33	Good	Slight to moderate	1 16		–	Improved on bed-rest alone, further improvement following intravenous therapy. Under therapy 4 months
18 (57 C M) Fair	Far advanced cavitation rt upper lobe	1 32	90–100 ml	+	Oral 4 gm q d Intravenous 50 mg B T W	84 48	Mod Good	Moderate		30–40 ml	–	Followed for 6 months since therapy started

* T I W 3 times weekly, B T W 2 times weekly



Fig 139—(Case 12 in Table 53) Ulcerating granulomatous lesion involving the major portion of the floor of the mouth Culture and biopsy revealed *H capsulatum* Ulcerative disease has destroyed both sublingual caruncles



Fig. 140—(Same case as in Fig 139) Complete healing of lesion after 3 weeks of intravenous amphotericin B A small fistulous track through which both submaxillary ducts empty can be seen to the right of the attachment of the frenulum

patients is presented in Table 54 It should be noted that Cases 13 and 14 received largely prolonged oral therapy Both these cases showed clinical improvement and slight to moderate radiographic change but sterilization of the sputum was obtained in Case 13 only The remaining 4 cases received oral and intravenous amphotericin B In all these cases clinical and radiographic improvement as well as sterilization of the sputa was accomplished In only one instance, however, was cavity closure noted Figs 141, 142, 143 and 144 demonstrate the typical pulmonary lesions and their response to amphotericin B

TABLE 54
CHRONIC PROGRESSIVE PULMONARY HISTOPLASMOSIS SUMMARY OF CLINICAL AND LABORATORY DATA ON CASES TREATED WITH AMPHOTERICIN B

Pre-treatment					Regime		Post-treatment					Comments
Case number and condition	Radiograph	C F titre	Sputum		Route, dose* and frequency	Number of doses	Clinical response	Radiograph improvement	C F titre	Sputum		
			Amount	Culture						Amount	Culture	
13 (42 W M) Fair	Far advanced cavitation bilaterally	1 32	30-60 ml	+	Oral 4-6 gm	330	Good	Slight to moderate	1 16	5-10 ml	–	Cavity (1 5 cm) right sub apex disappeared. Cultures negative for 9 months
14 (64 W M) Poor	Far advanced cavitation bilaterally	1 256	10 90 ml	+	Oral 4-8 gm q d Intravenous 100 mg q d	240 9	Fair	Moderate following intravenous	1 132	15-10 ml	+	Improved remarkably with intravenous drug, but received tetracycline at same time
15 (37 W M) Fair	Moderately advanced cavitation bilaterally	1 8	50-60 ml	+	Oral 4 mg q d Intravenous 50 mg T I W	50 31	Good	Marked	1 8	5-10 ml	–	Febrile response following intravenous injection daily Under therapy 4 months
16 (38 W M) Fair	Far advanced cavitation bilaterally	1 8	30-60 ml	+	Oral 4 gm q d Intravenous 66 mg T I W	50 31	Mod Good	Moderate		3-5 ml.	–	Under therapy 4 months 30 lb weight gain
17 (35 W M) Fair	Moderately advanced non cavity apical disease	1 64	15 20 ml	+	Oral 4-6 gm q d Intravenous 50 mg T I W	55 33	Good	Slight to moderate	1 16		–	Improved on bed-rest alone, further improvement following intravenous therapy Under therapy 4 months
18 (57 C M) Fair	Far advanced cavitation rt upper lobe	1 32	90 100 ml	+	Oral 4 gm q d Intravenous 50 mg B T W	84 48	Mod Good	Moderate		30-40 ml	–	Followed for 6 months since therapy started

* T I W 3 times weekly B I W 2 times weekly



FIG 141 —(Case 18 in Table 54) Chronic progressive cavitary histoplasmosis with marked involvement of the right upper lobe Prior to therapy.



FIG 142 —Same case as Fig 141 Considerable clearing in both lungs, more marked in the right, after oral amphotericin B (1 gramme q i d Nov 1–Dec 30, 1956, and Feb 28–Mar 16, 1957) plus IV amphotericin B 50 milligrams twice weekly Nov 5, 1956–Apr 11, 1957

Severe acute pulmonary histoplasmosis—A case of severe "epidemic" histoplasmosis was selected to observe the effect of the drug on the acute form of the disease There was a prompt clinical response with fall in temperature and rapid radiographic clearing which was closely coincident with intravenous administration of the drug (Fig 145) It should be cautioned that the course is difficult



FIG 143 —(Case 15 in Table 54) Bilateral apical histoplasmosis with cavitation just prior to treatment



FIG 144 —Same case as Fig 143 Marked reduction in parenchymal disease after 3 months' therapy with amphotericin B

to interpret since recovery is the rule in this form of histoplasmosis, but, on the basis of past experience, it is our opinion that this illness was significantly shortened

Dosage and administration.—The oral preparation used in these studies was administered as 200-milligram tablets of crystalline

amphotericin B Administration in dosages up to 8 grammes daily presented no problems; however, poor absorption from the gastro-intestinal tract makes this a questionable route of administration Temporary improvement of several cases while under oral therapy indicated that this drug even in extremely low levels exerts some *in vivo* fungistatic effect.

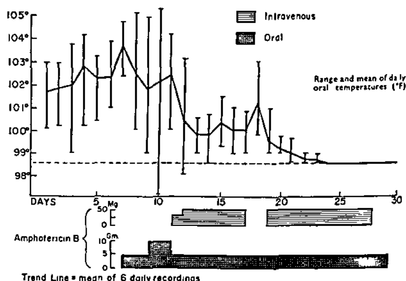


FIG 145 —Depicts the temperature curve and response of a case of severe acute pulmonary histoplasmosis to amphotericin B therapy Note rapid fall in temperature curve following intravenous amphotericin B

Intravenous amphotericin B used in this series of cases was administered as a colloidal suspension of estimated particle size 3 microns The desired dose of amphotericin B was suspended in a 5 per cent aqueous solution of dextrose in a concentration of 1 milligram of drug per 5–10 millilitres of water Administration of the drug over a 6-hour period greatly reduced the incidence of side reactions Studies by Louria, Feder and Emmons[18] indicated that amphotericin B serum levels of 0 7 to 1 5 µg per millilitre offered [illegible]

comparable levels were demonstrated in humans receiving a dose

equivalent to 1 milligram per kilogram body-weight. Levels above 0 1 µg per millilitre were found to persist for approximately 18 hours One case receiving 1·7 milligrams per kilogram body-weight maintained a serum level of 0·3 µg per millilitre for 30 hours after the drug was administered From this data it is evident that a dose equivalent to at least 1 milligram per kilogram body-weight is desirable The persistence of the drug in the serum with higher doses indicates that such doses could be given every other day Despite adequate amphotericin B serum levels, measurable spinal fluid levels (>0 06 µg. per millilitre) were not detected among the spinal fluids assayed

Toxic manifestations and side reactions—No evidence of either acute or chronic toxicity was noted in any of the patients receiving the oral form of amphotericin B Several of these patients received a total dose in excess of 1,000 grammes without ill effect In many cases receiving intravenous therapy supplemented by oral drug, minor upper gastro-intestinal symptoms and headache were noted

The colloidal suspension of amphotericin B administered intravenously to the cases in this series resulted in several rather consistent and undesirable side reactions In almost every instance, shaking chills followed by a "spike" in temperature, during administration of the drug, were noted It has been our experience that careful premedication with antihistamines and antipyretics will reduce these to a minimum. Chemical phlebitis was troublesome but its incidence was not prohibitive if the drug was given slowly over a 6-hour period In several instances the drug infiltrated into the surrounding subcutaneous tissues. Although this resulted in mild discomfort and redness at the site, no serious complications resulted

Repeated haematopoietic, hepatic and renal function studies failed to show any evidence of toxicity In only 1 patient was there any evidence of possible toxic effect In this case a transient rise in the blood urea nitrogen followed a dose of amphotericin B equivalent to approximately 1 4 milligrams per kilogram body-weight This may have been related to persistent vomiting due to increased intracranial pressure, and resultant dehydration Following rehydration the blood urea nitrogen returned to normal In this same patient the drug was discontinued earlier than planned because of the development of generalized pruritus

DISCUSSION

This study, conducted over the past several years, was designed to "screen" promising antifungal agents Clinical trials conducted on

amphotericin B Administration in dosages up to 8 grammes daily presented no problems; however, poor absorption from the gastro-intestinal tract makes this a questionable route of administration Temporary improvement of several cases while under oral therapy indicated that this drug even in extremely low levels exerts some *in vivo* fungistatic effect

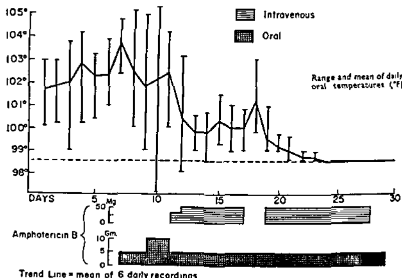


FIG 145 —Depicts the temperature curve and response of a case of severe acute pulmonary histoplasmosis to amphotericin B therapy Note rapid fall in temperature curve following intravenous amphotericin B

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SUMMARY

Experiences with the treatment of over 60 cases of deep mycotic infections with 7 antifungal agents are reviewed Favourable results in the treatment of cryptococcal meningitis and histoplasmosis with a new antibiotic, amphotericin B, are reported.

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38 patients with cryptococcal meningitis or progressive histoplasmosis with 2 hydroxystilbaminidine, aminostilbamidine, MRD-112, mycostatin, and amebacide were disappointing In only 1 patient was either clinical improvement or cultural conversion attained.

Among the patients treated with amphotericin B, preliminary results were dramatic, with both clinical improvement and cultural conversion Poor absorption from the gastro-intestinal tract makes the present oral preparation of questionable value. Studies are now in progress to find some means of enhancing absorption from the gastro-intestinal tract. The intravenous preparation is moderately well accepted by the patient in its present state and "adequate" blood levels are easily obtainable.

The problems of short follow-up periods and spontaneous remissions even among those forms of mycotic infections generally considered fatal must be considered. This may well account for the response noted in any single case treated with amphotericin B It is, however, unlikely that this can explain the consistently favourable response and conversion of cultures in all patients receiving a significant amount of intravenous drug It is difficult to explain the dramatic response obtained in cryptococcal meningitis in the light of our inability to demonstrate drug levels in the cerebrospinal fluid It may be that only small amounts of drug are necessary and the bioassay technique employed does not detect such levels *In-vitro* studies and clinical trials now proceeding in patients with blastomycosis and coccidioidomycosis indicate that this agent may well be a "broad spectrum" antifungal antibiotic

The moderate radiographic improvement noted among those with chronic progressive cavitary histoplasmosis is not surprising if one considers the underlying pathological changes The fact that marked reduction in sputum and cultural conversion was attained is considered to be a highly significant finding Recently we have followed some 30 cases of chronic progressive pulmonary histoplasmosis by frequent cultures for varying periods up to 2 years Of the 300 cultures performed, *H capsulatum* was recovered in 75 per cent of the specimens[15] It appears to us that the therapy of histoplasmosis with amphotericin B may well be in the same stage as that of tuberculosis in the early streptomycin era Combined drug therapy may be the solution to the problem[1]. Although surgery has been of definite value in appropriate cases, fear of complications with an organism resistant to therapy has been a major deterrent to this approach The availability of a specific therapeutic agent will undoubtedly make this approach more feasible

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